Climate change

and the epidemiology of gastrointestinal nematodes of sheep

by

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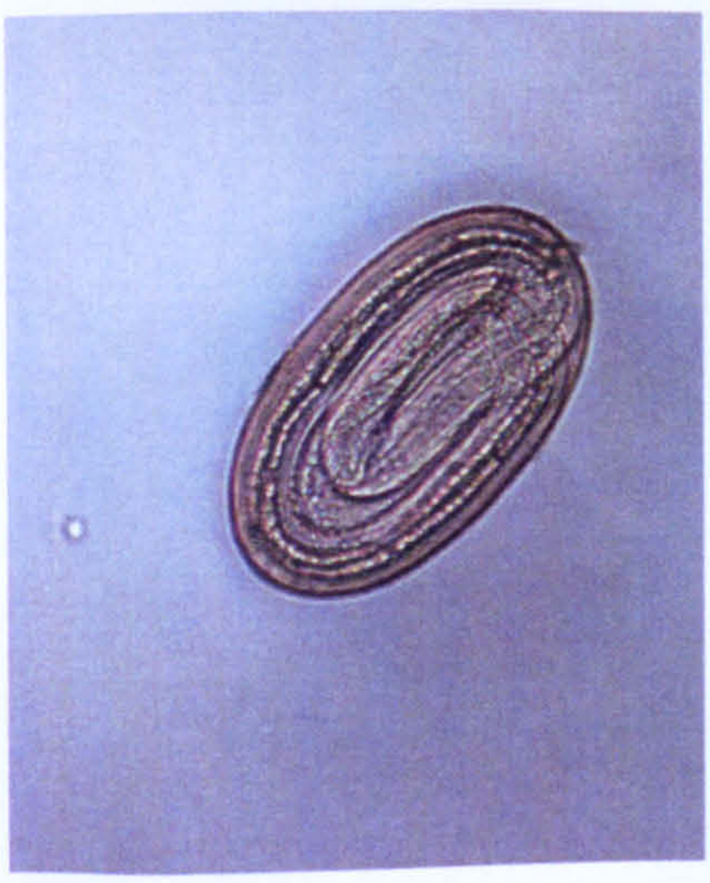
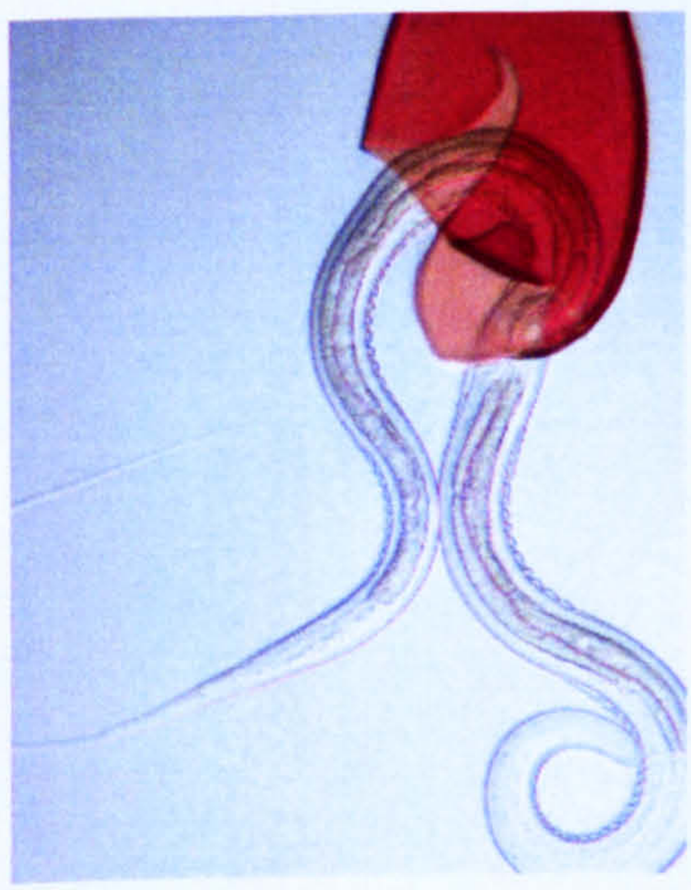
Interestingly, parasites which have abbreviated their life cycle to a permanent parasitic phase like to think of parasites with more complex life histories as 'disgusting little creatures'.

The specialists have as yet not completely lost their ability to respect and admire their peers but it may take them up to several years of intensive study to achieve such a state of mind.

JvD - May 2007





Hatching *N. battus* egg

Abstract

This thesis describes the beginning stages of the study of the effects of climate change on the epidemiology of gastrointestinal nematodes affecting sheep in the UK. It aims to
(i) contribute to our understanding of the immediate, and longer-term, effects of climatic factors on these parasites, with a view to devising worm control measures for the future
(ii) prioritise research efforts
(iii) explore strategies for the study of the effects of climate change on infectious pathogens.

A strategy of quantifying change that has already occurred is adopted. First, a 32-year surveillance data set on parasitic gastroenteritis incidence is explored for recent changes and UK regional differences. For the species *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *Haemonchus contortus*, generated hypotheses are subsequently tested making use of a simple, temperature-driven, R_0-based model. The model captures all changes in parasite abundance observed at pasture. Second, adaptations of a species which has moved from the Arctic to the temperate regions are studied. The vital rates of the species *Nematodirus battus* are described for the first time and stochastic mathematical models are devised.

The study not only finds evidence that global warming is already changing the epidemiology of these nematodes but also that small differences in temperature may account for significant inter-regional differences in epidemiology. Temperature thresholds for development and/or hatching are identified as key climate-related drivers of parasite abundance in the temperate regions. These thresholds do not appear to be highly adaptable but other traits timing the presence of infective stages at pasture (such as hypobiosis) are. A better understanding of these adaptable traits is vital for accurate predictions of future parasite epidemiology. For all species the force of infection is predicted to increase in autumn and therefore work on the immunology of older, partially immune, animals is also prioritised.

Acknowledgements

For over three years I had the honour of being Eric Morgan's first PhD student. I have no doubt that Eric has been the ideal supervisor for me. He gave me extensive freedom to follow up leads while stepping in at crucial moments, knowing where it was all going without dictating anything. Eric, I admire your sharp intelligence and in-depth knowledge of parasitology but perhaps even more your patience while dealing with less intelligent beings. For the future, I hope that I may continue to learn from you while working together, and that there will be more time for a few beers as well.

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Crucial to the completion of this thesis has been the free access to Lower Failand Farm nr. Bristol, run by the Amos family. On numerous occasions, for two whole years, Paul and Caroline got the lambs in for me, ready for sampling. Whenever I needed material I was made welcome, I was allowed to sample herbage, non-grazed plots were made available for experiments and I was invited in for coffee. Simply observing grazing animals and everyday farm life on a regular basis has helped me staying in touch with on-farm reality.

The counting of eggs and larvae of three *N. battus* isolates in chapter 5, and the numerous herbage larval counts in chapter 6, could simply not have been carried out without the fantastic help of Marieke de Louw and Leonie Kalis, two vet students from the Netherlands. The drive and enthusiasm of these ladies, who even gave up their weekends, was instrumental in finishing the laboratory work for both chapters.

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I very much wish to thank Matthew Baylis and Diana Williams for employing me before I had finished the thesis. Your exceptional kindness and patience during the write-up, at a time when I most needed it, has meant a lot to me.

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Many thanks to my mum for her everlasting support and for wanting to understand what I was doing. Last but not least thanks to my father for passing on an inquisitive mind. I hope you would have been proud.

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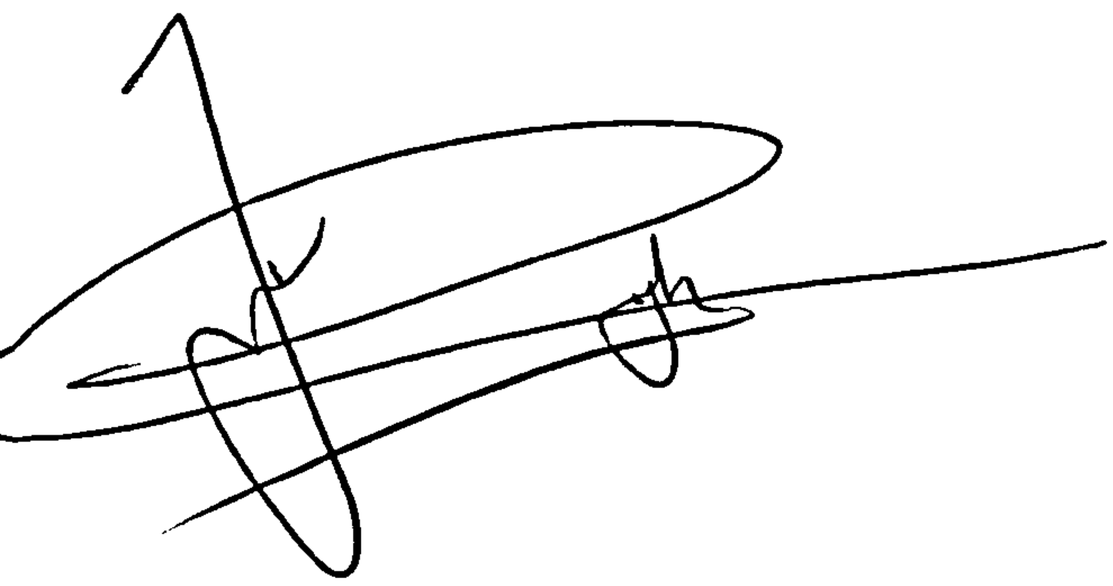
To Felix and Ruben,

two perfect boys,

born during the delivery of this thesis.

Author's Declaration

I declare that the work in this dissertation was carried out in accordance with the Regulations of the University of Bristol. The work is original, except where indicated by special reference in the text, and no part of the dissertation has been submitted for any other academic award. Views expressed in this dissertation are my own and not those of the University.



Jan van Dijk

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Chapter 1- Introduction

1.1 Background to the thesis

Climate change is probably the greatest long-term challenge facing the human race. Increasing global temperatures will bring changes in weather patterns and rising sea levels while altered rainfall patterns are predicted to increase the incidence of both drought and flooding events (Alcamo *et al.* 2007). Amongst the most important effects of these changes will be the emergence and spread of infectious diseases, including those of animals (Khasnis and Nettleman, 2005). Recent outbreaks of Foot-and-Mouth Disease, Avian Influenza and bluetongue are stark examples of the potential impact of emerging infectious diseases of animals on national economies, animal welfare, and human health.

The field of climate change and its effects on the dynamics of pathogens of veterinary importance has lagged behind climate change focussed research in other disciplines, to the extent that, in the Fourth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC), no animal pathogens are listed in the extensive overview of observed changes by Rosenzweig *et al.* (2007). Published work has focussed on the emergence of new diseases and disease vectors (e.g. Wittmann and Baylis, 2000; Colebrook and Wall, 2004; Purse *et al.* 2005; Gould *et al.* 2006) but effects on endemic disease have hardly been addressed by robust science. Veterinary workers have been urged to wake up to this fact in the veterinary press (Anon, 2007c).

Parasitic helminths of the class Nematoda (roundworms) are ubiquitous on livestock farms. Arguably every single ruminant farmed in the UK will be infected by one or more species of

this class at some stage in its life. If the level of infection is high this may lead to clinical parasitic gastroenteritis. This syndrome may have very significant consequences, both for animal welfare and in terms of economic losses. Exact numbers of animals dying from these infections can only be estimated. Based on submissions to the main UK network of surveillance laboratories (see chapter 2) a conservative estimate would be 5,000- 10,000 sheep in 2006 alone. Economically, subacute and chronic disease, with clinical signs such as anorexia, weight loss, diarrhoea, impaired reproduction, abortion and a reduced milk yield, and prophylactic treatment, represent even higher costs to the farming industry (Brunsdon, 1988; Ward, 2006; Louie *et al.* 2007).

The eggs of the nematodes involved have to develop into infective larvae, and migrate onto herbage, at pasture before transmission can occur. Assuming that hosts are present, the success rate of this non-parasitic phase is solely determined by climatic factors (as reviewed by O'Connor *et al.* 2006) and therefore these parasites are likely to be highly sensitive to climate change. Increases in mean daily temperature as small as 1°C are thought to have significant effects on entire ecosystems (Fischlin *et al.* 2007) and significantly increase development rates of nematodes (Kutz *et al.* 2005). Global warming therefore has the potential to increase parasite-related losses dramatically.

In recent years, repeatedly, important changes in the epidemiology of helminths have been reported in the UK. An increased incidence of parasitic gastroenteritis (e.g. Anon, 2004; Anon, 2005a; Anon, 2006a) has been signalled by the veterinary surveillance network. The incidence of fasciolosis, caused by the fluke *Fasciola hepatica* (e.g. Anon, 2007d; Pritchard *et al.* 2005), and dictyocaulosis by the bovine lungworm *Dictyocaulus viviparus* (David, 1997; van Dijk, 2004) appears to have increased significantly over the same period of time.

Expansion of parasite transmission windows into the Autumn has also been described (Anon, 2005b; Anon, 2007a). At the same time different ages, and species, of animals have been affected by clinical disease. The helminths involved have traditionally caused disease in immunologically naive young animals but increasingly adult animals appear to be affected (van Dijk, 2004; van Dijk and Morgan, 2006; Sargison *et al.*2007). Acute fasciolosis, associated with sudden death, is normally only witnessed in sheep but was recently also described in calves (Anon, 2007b) and adult pigs (Anon, 2006b). Disease resulting from infections with *Haemonchus contortus* (Anon, 2006c) and *Fasciola hepatica* (Pritchard *et al.* 2005) has emerged in regions where these parasites were thought to be of no clinical importance, suggesting a spatial expansion of their transmission range. All these changes suggest increases in force of infection at pasture. Climate change may already affect parasite epidemiology.

Concurrently, effects of the changes described above are compounded by the rapid development and spread of anthelmintic resistance (as reviewed by Jabbar *et al.* 2006), which poses a direct, ever increasing, threat to the sustainability of sheep farming in large parts of the UK. The rate at which anthelmintic resistance develops has been shown to be strongly influenced by the proportion of the parasite population that is not exposed to anthelmintic treatment (e.g. the population outside of the host at the time of treatment, the population *in refugia*; Martin *et al.* 1981; Sissay *et al.* 2006). As climate change is likely to alter this proportion, for example as the result of droughts (Papadopoulos *et al.* 2001), it may well influence the build-up of anthelmintic resisitance.

In conclusion, climate change has the potential to increase the transmission intensity of highly pathogenic, ubiquitous, parasites to levels uncontrollable by current management strategies. However, currently, research in the field has not progressed past describing perceived changes. There is an urgent need to quantify changes in parasitic challenge to animals to be able to inform veterinarians and farmers on how best to control helminth disease in food producing animals in the coming decades.

Macro parasites are likely to be early indicators of climate change (Baylis and Githeko, 2006). Most micro parasites, although perhaps not as direct in contact with the environment, also have to survive and/or replicate in between infection of hosts. Thus, macro parasites may provide early models for the response of these infectious pathogens to climate change. Vector borne viruses, for example, have over-winter strategies very similar to parasitic nematodes, exploiting both the host and the vector (Purse *et al.* 2005). Increased temperatures may alter the death, and replication, rates of virus in the vector and expand windows of opportunity for virus replication.

In a wider ecological sense, the new opportunities and threats created by climate change are very similar for many species on earth. Most species will encounter changing windows of opportunity for development and will have to adapt over-wintering strategies. For example, diapause may no longer be the best strategy for insects (Musolin, 2007). Principally, seeds in seed banks, which may or not delay their development to the next growth season (Valleriani and Tielborger, 2006), face the same choices as nematode parasites infecting a host in autumn.

1.2 The parasites

1.2.1 Life cycle and climatic influences

Trichostrongyloids are essentially parasites of the stomach and intestine of their hosts (Anderson, 2000). The most pathogenic species parasitising sheep live in either the ovine abomasum or the small intestine (Coop and Angus, 1973; Barker, 1975; Taylor and Pearson, 1979). The lifecycle of Trichostrongyloidea is direct. Ellipsoidal ova pass to the ground with the faeces of the host, hatch into filariform (L1-stage) larvae, and develop through larval stages L2 and L3 in the faeces. The infective L3-stage larva then migrates out of dung, onto surrounding vegetation, where it may be ingested. The subfamily of Nematodirae express an important modification to this standard lifecycle (see figure 1.1); their L1, L2 and L3-stage larvae all develop in the egg and on hatching of this egg the freed larva is immediately infective (Thomas, 1959a). The typical pre-patent period of the Trichostrongyloidea is 2-3 weeks (Dunn, 1978).

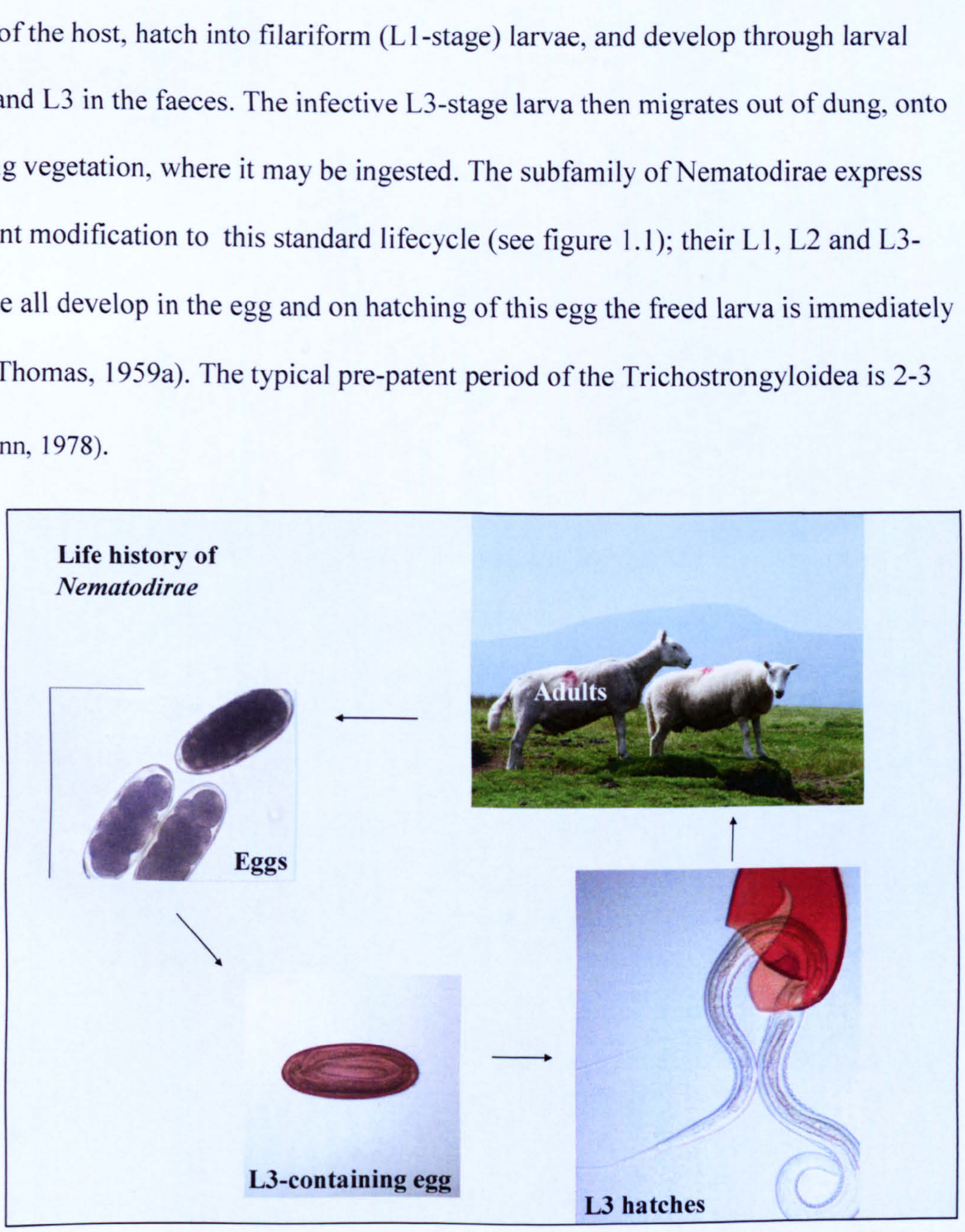


Figure 1.1 The *Nematodirus* life cycle

Trichostrongyloid species affecting sheep in the UK belong to four different subfamilies: Nematodirae (*Nematodirus battus, N. filicollis*), Ostertaginae (*Teladorsagia circumcincta*), Trichistrongylinae (*Trichostrongylus colubriformis*, *T. axei*, *T. vitrinus*) and Haemonchinae (*Haemonchus contortus*). Some important characteristics of the subfamilies are characterized in table 1.1. The species have distinct differences in origin and epidemiology

	Nematodirus spp.	*Trichostrongylus* spp. and *Teladorsagia circumcincta.*	*Haemonchus contortus*
Origin	Arctic (Hoberg, 2005)	*T. circ*: Arctic (Hoberg *et al.* 1999) *Trichs*: Not known	Africa (Hoberg *et al.* 2004)
Life cycle	L1/L2/L3 in egg; L3 hatches after stimulus only (Christie, 1962)	L1 hatches when developed (Salih and Grainger, 1982)	L1 hatches when developed (Levine and Todd, 1975)
Host specificity	Low: ruminants and rabbits (see chapter 5)	Low: ruminants and rabbits (Hoste *et al.* 1998)	Low: ruminants (Lichtenfels *et al.* 1997)
Most pathogenic stage	Burrowing larvae (Coop and Angus, 1973)	Larvae and adults (Blanchard *et al.* 1986; Taylor and Pearson, 1979)	Adult worms (Dunn, 1978)
Fecundity	40 eggs/female/day (Coyne *et al.* 1991; Mapes and Coop, 1972)	200 eggs/female/day (Gordon, 1948)	5,000-10,000 eggs/ female/day (Gordon, 1948; Levine and Todd, 1975)
Worm generations per year (UK)	1 (Gibson and Everett, 1981)	1-3 (Boag and Thomas, 1975b, 1977; Uriarte *et al.* 2003)	1-3 (Uriarte *et al.* 2003; Waller *et al.* 2004)

Table 1.1 Some differences and similarities between economically important Trichostrongyloid species pathogenic to farmed ruminants.

and may therefore provide different models for responses to climate change. Differences in responses of their free-living stages to climatic variables are summarized in table 1.2. In summary, *Nematodirus battus* appears to be an Arctic-adapted species, trading off excellent protection of larvae against environmental noxes with low fecundity and few worm generations. However, many parameters have not been researched for this species. At the other end of the spectrum, *Haemonchus contortus* is tropical-adapted and appears to compensate relatively high weather-related losses, and a relatively small window during which development is possible, with a very high fecundity. *Teladorsagia circumcincta* and *Trichostrongylus* species have a relatively long development season, are relatively long-lived, more resistant to desiccation than *H. contortus*, and have a fecundity somewhere in between the other two species.

1.2.2 Over-winter survival

Differences in epidemiology of the three species groups presented in table 1.1 and 1.2 are perhaps best summarized by their distinct over-winter strategies (Thomas and Waller,1979):

1) Over-wintering of the population depends mainly on eggs surviving at pasture - *Nematodirus* spp. (Thomas and Stevens, 1960).

2) Late summer/autumn-developed L3 stage larva survive at pasture, while a different part of the population survives, as inhibited L3 stage larva, in the host– *Teladorsagia circumcincta, Trichostrongylus spp.* (Eysker, 1978 & 1981; Jacobs and Rose, 1990).

3) Over-winter survival solely as inhibited L4 stage larva in the host- *Haemonchus contortus* (Boag and Thomas, 1977; Thomas and Waller, 1979; Waller *et al.* 2004).

	Nematodirus spp.	*Trichostrongylus* spp. and *Teladorsagia circumcincta*	*Haemonchus contortus*
Egg development	In soil (Gibson and Everett, 1981)	In dung (Levine and Anderson, 1973)	In dung (Levine and Todd, 1975)
Optimum temperature egg development	Not known	25°C (Wang, 1967)	20-30°C (Berberian and Mizelle,1957; Hsu and Levine, 1977)
Minimum temp. for egg development	Not known	4-5°C (Wang, 1967; Beveridge *et al.* 1989)	10°C (Gibson and Everett, 1976a)
Minimum Relative Humidity for egg development	Not known	76-85% (Wharton, 1982; Hsu and Levine, 1977)	95% (Hsu and Levine, 1977)
Effect of desiccation on eggs	No development (Parkin, 1976)	Dead after 48 hours at 70 % RH (Waller and Donald, 1970)	All dead after 48 hours at 86% RH (Rose, 1963; Waller and Donald, 1970)
Minimum hatching time	Months (Parkin, 1976; Gibson and Everett, 1981)	19 hours, at 20°C (Monnig, 1926)	16 hours, at 26 °C (Veglia, 1916)
Sensitivity L1/L2 to desiccation	Protected by egg shell (Thomas, 1959a)	Dead within 6 hours at 95% RH (Wharton, 1982)	All dead within 24 hours below 100% RH (Rose, 1963)
Minimum time L3 development	5-6 weeks at 20°C (Parkin, 1972)	3-4 days at 25°C (Ciordia and Bizzel, 1963; Rose and Small, 1984)	60 hours at 33°C (Berberian and Mizelle, 1957)
Sensitivity L3 to desiccation	Highly resistant (Parkin, 1976)	Less sensitive than *Haemonchus* (Levine and Todd, 1975)	Sensitive (Shorb, 1943; Todd *et al.* 1976, Gibson and Everett, 1976a)
Resistance L3 to freezing	Highly resistant (Ash and Atkinson, 1986; Rose and Jacobs, 1990)	Resistant to repeated freezing and thawing (Jasmer and Wescott, 1987)	Sensitive to single frosts and especially repeated freezing/ thawing (Jasmer and Wescott, 1987)
Resistance L3 to high temperatures	Not known	At 32°C (and 100% RH) all dead within a week (Unpublished observation)	Survives at least 35 days at 36°C (Shorb, 1943)
Maximum survival L3 at pasture	2 months (Thomas and Stevens, 1960; Graham *et al.* 1984; Thomas, 1991)	Up to 6 months (during autumn/winter) (Barnes and Dobson, 1987)	Up to 5 months (Besier and Dunsmore,1993)

Table 1.2 Climate-related characteristics of the free-living stages of the species under study.

Hypobiosis (Michel, 1974; Michel *et al.* 1976) is the trait of inhibition of development as freshly ingested L3-stage, or L4-stage, larva in autumn, followed by the completion of development into adult worm at a later date. Cues for inhibition are thought to be immunity of the host (e.g. Chapman *et al.* 2002; Smith *et al.* 2007) and environmental influences on L3 at pasture. A variety of environmental cues has been associated with arrested development, including temperature (Jacobs and Rose, 1990; Fernandez *et al.* 1999), photoperiod (Lutzelschwab *et al.* 2005), and drought (Giangaspero and Bahhady, 1992; Ndamukong and Ngone, 1996). The influence of the same environmental cues can have contrasting effects in different geographical locations, suggesting that the trait is highly adaptable to local climatic conditions. For example, Langrova and Janskova (2004), working in the Czech Republic, found increased proportions of larvae arresting at decreasing photoperiods whereas Lutzelschwab *et al.* (2005), in Argentina, found that the capacity for developmental arrest was lost under such circumstances. Hypobiosis appears targeted towards surviving periods of adverse conditions at pasture, whatever these represent locally, as shown by its absence in areas where development and transmission are secured all-year-round (El-Alzazy, 1990; Waruiru *et al.* 2001). Interestingly, Langrova and Janskova (2004) suggested that the proportion of larvae arresting may also depend on the effects of these environmental cues on the hosts.

The main trigger for resumption of development is thought to be temporary diversion of energy investments of highly pregnant animals from the immune system into offspring (Medley, 2002) leading to a very significant peri-parturient rise in worm egg counts of ewes (e.g. Temebely *et al.* 1998; Nganga *et al.* 2004).

1.3 Host-parasite interaction

1.3.1 Dependence on adult hosts for persistence

Dorset ewes are fertile throughout the year but virtually all other sheep breeds only mate around autumn time. Sheep farms therefore normally have one crop of lambs per year, and UK lambs are, on the whole, born in the period February-May. Lambs are slaughtered from the age of 4-6 months onwards. Thus, on late-lambing farms, and farms fattening 'store lambs', older lambs may be present at pasture during autumn and winter but on many farms lambs will have gone to slaughter around the start of autumn. This means that often, during autumn and winter, only ewes, and ewe-lambs (replacement ewes), will be present on the farm. The different over-winter strategies of the worm species described above therefore also reflect differences in dependence on adult hosts for their year-to-year persistence.

Nematodirus battus is thought to have a lamb-to-lamb epidemiology, independent of adult hosts (Gibson and Everett, 1981). *T. circumcincta*, and *Trichostrongylus* spp., depend on both the environment and the adult host for survival (Smith and Archibald, 1965; Thomas and Waller, 1979) while the persistence of *H. contortus* depends solely on adult sheep and the peri-parturient rise (Waller *et al.* 2004).

1.3.2 Age-related immunity

Although adult, non-pregnant, ewes may have a lower tolerance threshold for chronic worm infections (Behnke *et al.* 1992; Medley, 2002) it is well established that low-level infections with *T. circumcincta*, *Trichostrongylus s*pp. and *H. contortus* do occur (e.g. Smith, 2007).

Adult animals may even show clinical signs of infection, normally associated with the peri-parturient rise (Sargison *et al.* 2007; van Dijk and Morgan, 2006). However, amongst veterinary parasitologists, the well-established hypothesis is that mature sheep are refractory to *Nematodirus battus* infection. The existence of such an age-resistance phenomenon remains controversial (Winter, 2002). The work most referred to in support of the theory is that of Gibson and Everett (1963), who infected two sheep (30 months of age) with 300,000 L3 and slaughtered one sheep one month later and the other two months later. From experience (Gibson, 1959a) Gibson and Everett knew lambs 6 weeks of age infected with 300,000 L3 fell severely ill or died weeks after infection. Since the older animals showed no signs of disease this was interpreted as age-resistance. The post mortems revealed establishment rates of 40% and 19% one and two months post infection, respectively. Winter *et al.* (1996) showed that some eight-month old lambs were virtually refractory to infection unless treated with immunosuppressants. However, at a low dose of 50,000 L3 administered, the slaughter of some non-treated lambs revealed establishment rates of 10-25%. Israf *et al.* (1997) found lower establishment rates in animals infected at 32 weeks of age than in animals challenged at 21 or 26 weeks of age. However, the proportions of larvae establishing in older animals described in these three studies would, in studies looking at infections in younger lambs (Taylor and Thomas, 1986; Israf *et al.* 1996), have been classified in the 'low-responder' category of animals not mounting a strong immune reaction. The presence of thousands of adult *N. battus* worms has been described in older, clinically healthy, lambs infected at pasture (Rickard *et al.* 1987).

In summary there is no clear evidence that age-related resistance, an inherent resistance related to age or the size of an animal, exists. It appears that an absence of clinical signs of disease has been confused with resistance to infection.

1.4 Approach to the study

Climate change may have short-term effects on parasites which in turn will lead to parasite adaptation (Poulin, 2007; Skelly *et al.* 2007). At the same time, it may have effects on hosts and, in the case of farmed animals, on host management. These layers of complexity make it hard to predict the longer-term future accurately. As a starting point, it was decided to focus on the influence of climate change on the free-living stages of the species described above. One of the more obvious conclusions of the UK Government Foresight Project 2006 on infectious diseases was that "We must understand how climate affects infectious diseases today before we can predict climate change's impacts of the future" (Brownlie *et al.* 2006). The bridging of gaps in our understanding of the effects of temperature and rainfall on the free-living stages of the nematodes under study therefore forms an integral part of the study of future climate change and large parts of this thesis are dedicated to it.

The first papers on possible effects of global warming on parasite development have emerged (Kutz *et al.* 2005; Poulin, 2006). However, in the temperate regions, it is not clear whether increases in development rates will translate into increased parasite abundance. As may be clear from table 1.2, climate change is likely to have contrasting effects on different stages of the life cycle. For example, increased mean temperatures may increase egg

development rates but increase larval death rates at the same time. Also, the development phase is bound by certain thresholds and warming will only increase development rates at above-threshold temperatures. Therefore, it appears impossible to predict the effect of global warming on parasite abundance without the use of mathematical models. The laboratory experiments part of this study are aimed towards the development of these models.

To date, there have been few published attempts to develop quantitative predictions of how disease incidence might be affected by climate change. Mathematical models have been applied to vector-borne diseases (e.g. Hay *et al.* 2002, Purse *et al.* 2007) but not to parasites with lifecycles that involve free-living stages. Existing models have explored the influence of increases in temperature but largely ignored changes in rainfall patterns. It is not known whether such abbreviations are justified in models of the epidemiology of parasitic nematodes. Also, even though adaptations of parasite behaviour, in response to changes in their environment, will impact heavily on the longer-term suitability of existing control measures, no model has, as yet, included parasite evolution.

Although not for all species introduced above, very detailed deterministic models already exist for *Teladorsagia circumcincta* and *Haemonchus contortus* (as reviewed by Smith and Grenfell, 1994). However, models of the effects of climate change on the free-living stages will have to include an element of climate uncertainty. Also, in particular to explore likely pathways of parasite evolution, parameter uncertainty will have to be modelled and analysed (Morgan *et al.* 2004). Therefore models need to be stochastic and preferably include few parameters.

A recent meeting on animal diseases and global warming heard professor D.J. Rogers (as quoted by Anon, 2007e) express his worry that, as future predictions cannot be disproved until the time comes, the work on models of responses of infectious diseases to climate change may reverse the trend of scientific enquiry. This seems a real danger indeed and the present study wants to investigate ways to increase confidence in predictions. One strategy serving to improve such confidence could be to compare predictions with documented changes that have already occurred. Thus, the study of historic changes, and existing adaptations of parasites to different climatic environments, appears very much part of a study of possible changes to take place in the future.

The next chapter therefore starts with an analysis of 30-year clinical parasitic gastroenteritis incidence data. Trends discovered are, for *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *Haemonchus contortus*, subsequently tested for correlation with the predicted influence of recent changes in temperature on the free-living stages. Attention then turns to a parasite that, since its introduction into the UK approximately 60 years ago, has had to adapt to extensive changes in climatic conditions. The basic vital rates of *Nematodirus battus* are documented and options for adaptation investigated. After a description of the influence of free water on development, hatching and migration a first simulation model of parasite ecology and epidemiology can be assembled. Model predictions are, once again, held against trends uncovered from disease incidence data.

1.5 Aims and objectives

This thesis wants to contribute to the beginning stages of the study of the effects of climate change on parasites of farmed animals. It aims directly towards the development of sustainable worm control measures for the farming industry and towards informed directions for future research. At the same time it explores the scope for putting the study of parasitism in a changing world into a wider ecological context. Specific aims are:

1) to enhance our knowledge of the effects of temperature and rainfall on the free-living stages of economically important parasitic nematodes of ruminants
2) to identify the key parasite traits likely to determine parasite abundance, and parasite evolution, under changed climatic conditions
3) to identify parasite traits likely to adapt to climate change
4) to investigate whether parasites are sensitive models for the wider study of climate change on living organisms, infectious pathogens in particular
5) to develop methods producing testable hypotheses and enhancing trust in predictions of the future.

Chapter 2 – Back to the future – developing hypotheses from historic data

2.1 Introduction

Although the influence of climate on the development and mortality of the free-living stages of nematodes of grazing ruminants has been extensively studied (Kao *et al.* 2000, O'Connor *et al.* 2006), and climate change might be expected to affect parasite transmission, there is little published evidence for recent changes in epidemiology. This is likely to, at least in part, be the result of a lack of reliable historic data, a quantified baseline, against which perceived changes can be held. Great Britain (GB) is quite unique in the way a network of veterinary surveillance laboratories has recorded disease outbreaks over a long period of time. In this chapter, national and regional trends in the reported incidence of gastrointestinal nematode disease in sheep are analysed using the 30-year Veterinary Investigation Diagnosis Analysis (VIDA) surveillance database.

The VIDA database records diagnostic submissions to a network of veterinary surveillance laboratories all over Great Britain, which has been in operation since 1975. From recorded data monthly and annual reports on diseases and disease trends are published (e.g. Anon, 2005a, Anon, 2006a, DEFRA 2007a). However, detailed analyses of disease trends over longer periods of time, or at a regional level, are not routinely conducted. Given regional variation in climate, it is not clear whether predictions of parasite epidemiology based on national climate data are appropriate, or if not at what spatial scale such predictions should be made (Learmount *et al.* 2006).

VIDA documents three categories of parasitic gastroenteritis (PGE) in sheep: 'Nematodirosis', 'PGE - worm species not otherwise specified' (NOS) and 'Haemonchosis'. As *H. contortus* is the only *Haemonchus* species reported in sheep in Great Britain, Haemonchosis refers to this species only. Nematodirosis could include several species, but in practice clinical submissions are dominated by *Nematodirus battus* and disease due to *N. filicollis* and *N. spatigher* has not been described in recent years. The vast majority of diagnoses in the 'NOS' category involve infections with *Teladorsagia circumcincta*, *Trichostrongylus colubriformis*, *Tr. vitrinus* and *Tr. axei*. The three categories therefore represent contrasting epidemiology as well as taxonomy. Thus, *Nematodirus* spp. persist from year to year largely as eggs on pasture (Gibson and Everett, 1981; Thomas and Stevens, 1960), and the effects of climate change are likely to be concentrated on hatching behaviour. Species in the 'NOS' category survive the winter as larvae on pasture, and also as arrested larvae and adults in the sheep (Thomas and Waller, 1979). Rising temperature would be expected to accelerate development and, given the low development threshold of 4-10°C (Beveridge *et al.*, 1989; Gibson and Everett, 1967; Young *et al.*, 1980), could enable development in winter. The free-living stages of *Haemonchus contortus* are relatively susceptible to low temperature, and survival through the winter in the UK is assumed to rely on persistence in the host. The lower threshold for development is 10-12°C (Besier and Dunsmore, 1993; Gibson and Everett, 1976). Moisture is generally considered not to be limiting to transmission of any of these species in the UK, although drought may prevent development and increase mortality of the free-living stages.

Analysis of temporal trends in parasite epidemiology using VIDA data is limited by the fact that this is a passive surveillance system (Thrusfield, 2005). As such, factors that influence farmer and veterinarian motivation to submit samples, as well as changes in laboratory methods and disease awareness, will confound results. However, PGE in sheep is somewhat unique in that data have been collected not only over a long period of time but also with little or no change in diagnostic methods. As a well known and long established endemic disease complex with no major changes in control methods or available therapy over the past few decades, submission of samples for PGE can also be assumed to reflect background disease incidence. However, because diagnosis incurs a monetary fee, farm economics are likely to influence submission rate. This chapter addresses this source of bias by comparing overall submission rate with the size of the national flock, and the value of lambs at slaughter, over the study period.

The aims of the analyses were to determine: (1) whether the incidence of PGE in sheep, as inferred from diagnostic submissions, has changed over the past 32 years; (2) whether there are detectable regional differences in seasonal patterns of disease, and hence whether national surveillance is sensitive to changes in epidemiology at a local scale; (3) the likelihood that climate change, in particular higher temperatures and drier summers, is already having an effect on nematode epidemiology in Great Britain; (4) whether such effects, if present, can help us to predict the future impact of climate change on the epidemiology of PGE.

2.2 Materials and Methods

The VIDA database records every submission made to the, currently, 17 regional laboratories of the Veterinary Laboratories Agency (VLA) in England and Wales, and the 8 laboratories of the Scottish Agricultural College (SAC) in Scotland. Before 1999 clinical cases of PGE were entered into this database based on the personal judgement of expert veterinary pathologists. Since 1999 diagnoses have been defined by centrally agreed criteria. These criteria, which are given in table 2.1, were based on what had been previous practice in the regional laboratories. Data for the diagnostic categories 'nematodirosis', 'NOS' and 'haemonchosis', were obtained from the Centre for Epidemiology and Risk Analysis (VLA Weybridge). For the first two categories data were available for the whole period 1975-2006, but for Haemonchosis records began only in 1989. Since the location of farms submitting

PGE Category	Case definition
Nematodirosis	Clinical history and/or gross pathology and/or histopathology, and either a) detection of significant numbers of adult or larval worms in the gastrointestinal tract or b) detection of characteristic eggs in the faeces
Haemonchosis	Clinical history and/or gross pathology and/or histopathology, and either a) detection of adult worms on the gastrointestinal tract or b) larval culture and identification of infective larvae
NOS*	Clinical history and/or gross pathology and/or histopathology, and either a) detection of adult worms in the gastrointestinal tract or b) a high faecal worm egg count or c) larval culture and identification

Table 2.1 Case definitions of the three different PGE categories entered in the VIDA database. In practice, faecal egg counts would be considered high above 8,000 per gram in haemonchosis, and 2,000 per gram in NOS (* PGE, worm species not otherwise specified).

samples was recorded, it was possible to obtain data for each of the separate counties in England, Wales and Scotland. However, the number of submissions from separate counties was too low to perform any meaningful analysis and therefore a regional approach was adopted. The relative contribution of each region to the total number of submissions is listed in table 2.2. From these eight regions, the five with the highest numbers of sheep sample

Region	submissions	% of total
Channel Isles	1,174	0
SouthWest	54,118	13
South East	29,225	7
Wales	74,187	17
Midlands	56,072	13
East	18,192	4
Northern	44,625	10
Scotland	148,827	35
Unknown	4,924	1
Total	431,344	100

Table 2.2 Contributions of the different regions to the total of diagnosable submissions, 1975-2006.

submissions were chosen for further analysis. The month of submission has been recorded in the VIDA database, but not the exact date. Statistical analysis used SPSS (version 12.0) software (SPSS Inc., Chicago, USA) as follows:

2.2.1 Number of diagnostic submissions

Absolute numbers of PGE diagnoses may yield useful information on disease trends but are confounded by factors such as the size of the sheep population, which varies by region and

over time, and the willingness of farmers to invest in disease investigation. These problems are attenuated when cases of PGE are expressed as a proportion of the total number of submissions received, and this forms the basis of most of the analyses. However, the size of the denominator (i.e. total submissions) and relative uptake of different tests then become important potential sources of bias. In order to take account of this, trends in the total number of sheep samples submitted annually to the contributing laboratories, and the number of submissions per head of sheep in the national flock, were examined using Spearman rank correlation against year. This analysis was extended to include the price of lamb in order to exclude a dominant effect of farm income on submission rates. Detailed information on all parameters was available for 1990-2005 only, and the year of the Foot and Mouth Disease epidemic (2001) was omitted as a clear outlier. UK Census data on the total numbers of sheep, the total area of grassland available for grazing, and the total number of holdings on which sheep are kept were obtained from the Farming Statistics Department of the Department for Environment, Food and Rural Affairs (DEFRA), and used to assess the likely effect of large scale changes in the structure of sheep farming on submission rates.

2.2.2 Overall UK trends 1975-2006

For each category of nematode infection, the sum of the number of diagnoses reached in England, Wales and Scotland was expressed as a percentage of the total of submissions received minus the submissions where a diagnosis was not applicable, for each of the years 1975-2006. Data were analyzed for significant correlation with year using Spearman rank correlation. In order to determine more precisely the timing of any changes, the proportion

of diagnoses in each year attributed to each nematode category was arcsine transformed and divided into six blocks of five years (1977-1981, 1982-1986, 1987-1991, 1992-1996, 1997-2001 and 2002-2006). The five-year blocks were compared in a one-way ANOVA followed by Tukey's *post hoc* test. For Haemonchosis the data allowed a comparison of just three five-year blocks (1992-1996, 1997-2001 and 2002-2006).

2.2.3 Regional trends 1975-2006

For each category of nematode infection, the total number of diagnoses reached in the regions Southwest, Midlands, Wales, North and Scotland was expressed as a percentage of the total of diagnosable submissions received minus the submissions where a diagnosis was not applicable for each of the years 1975-2006. Data were analysed for significant correlation with year using Spearman rank correlation.

2.2.4 Regional differences in rates of diagnosis

The annual number of PGE diagnoses as percentage of diagnosable submissions was compared for each region and nematode category for 1977-2006 using the Kruskal-Wallis test. Given the large sample sizes the Monte Carlo option in SPSS was chosen using 10,000 repetitions. Regions were ranked in order of highest to lowest percentage and the order assessed using the Jonckheere-Terpstra test (Pirie, 1983). *Post hoc* Mann-Whitney tests were run on data significant in the Kruskal-Wallis test, with the Bonferroni correction applied. In order to minimise the number of *post hoc* tests the smallest difference between mean ranks

of different regions was tested for significance, then the next smallest, etc. At the first significant result *post hoc* testing was halted and it was assumed that, as differences between the remaining groups were larger, these were also significant. Alpha was set at 0.05.

2.2.5 Seasonal patterns

In order to examine the typical seasonal distribution of disease, the number of diagnosed outbreaks per month was expressed as the percentage of the total number of diagnoses in that category over the whole year, for each of the years 1977-2006. To describe between-year variation, the monthly median percentage over this period was calculated with its inter-quartile range. For each nematode category three regions of particular interest were chosen for analysis. In all cases the Southwest and Scotland were chosen to represent the climatic extremes of Great Britain. For 'nematodirosis' and 'NOS', Wales was chosen as an intermediate region, and for haemonchosis the Midlands was chosen as the region in which *Haemonchus* has apparently been most successful according to previous analyses.

The proportions of annual cases occurring in each month were arcsine transformed and compared using one-way ANOVA followed by Tukey's test. From this analysis, months of peak abundance were identified by scanning for clusters of months in which the relative abundance was significantly higher than in all the other months. The peaks generally spanned three (nematodirosis), four (NOS) or two (haemonchosis) consecutive months. As a measure of the temporal concentration or 'peakedness' of the annual appearance of disease in each category, for each year the total proportion of cases diagnosed in the peak months

was calculated, regardless of exactly which calendar months these comprised. The resulting proportions were arcsine transformed and regions compared by one-way ANOVA and Tukey's test. The proportion of annual diagnoses for each category and region was also tested for correlation with time, using Pearson's correlation coefficient, and divided into six blocks of five years for comparison by ANOVA as already described. Finally, for nematodirosis the relative importance of autumn disease was assessed by summing the percentage of diagnoses in the months September, October and November for each year and region and comparing the arcsine transformed percentages in a one-way ANOVA.

2.2.6 Changes in seasonal patterns 1977-2006

a. Peak shifts

In order to assess whether typical seasonal patterns in disease have changed over the past 30 years, the months of the year were each given a number 1-12 (1 for January, etc.), and for each year the mean month of peak occurrence, *M*, was calculated as follows:

$$M = \frac{\sum_{i=a}^{i=b} p_i . n_i}{\sum_{i=a}^{i=b} p_i} \qquad \text{Equation 2.1}$$

where p_i is the proportion of annual diagnoses occurring during each of the months from the month prior to the peak period (as defined above), a, to the month following the peak period, b, and n_i is the number (1 to 12) of those months.

The significance of differences in the timing of the peak between regions was, for the categories nematodirosis and NOS, tested in a one-way ANOVA on *M* followed by Tukey's tests. In order to assess temporal shifts in the timing of peaks within regions *M* was tested for correlation with year using Spearman rank correlation.

b. Changes in relative importance of months over time

Positive correlations with year indicate a rising trend but say nothing about when that trend began. For this reason the years 1977-2006 were divided into five-years blocks as described above and for each nematode category the monthly proportions of total annual diagnoses were compared across blocks using the Kruskal-Wallis test. Positive or negative trends across blocks sequential in time were also assessed using the Jonckheere-Terpstra test. Only if the Kruskal-Wallis test for a certain month gave a significant result *and* the Jonckheere-Terpstra test indicated a significant positive trend, then the following *post hoc* Mann-Whitney tests were performed: (i) if the mean rank of the years 1997-2001 was higher than that of all of the four groups of years before 1997 then the years 1997-2001 were tested against the five-year block with the next highest mean rank, and (ii) similarly, if the mean rank of the years 2002-2006 was higher that of all of the five blocks before 2002 then the years 2002-2006 were tested against the block with the next highest mean rank. Bonferroni correction was applied where *post hoc* tests were carried out. Alpha was set at 0.05. There were too many months with no diagnosis of haemonchosis to perform a meaningful analysis on separate regions, therefore regional analyses were carried out for the categories 'nematodirosis' and 'NOS', and an overall UK analysis for 'haemonchosis'.

2.2.7 Climatic trends

Although the present analysis does not test specific associations with climatic data, trends in climate are relevant and are therefore broadly described. Mean monthly UK temperatures and the total monthly millimetres (mm) of rainfall for the period 1975-2006 were obtained from the UK meteorological office. In order to detect changes in these parameters over the past ten years, the 1975-1996 average was compared with that of 1997-2006. Over the whole 32 year period changes in each month were assessed using Spearman rank correlation with year. When trends were significant the most recent year was omitted from the series and the analysis repeated. If the analysis still produced a significant result another year was omitted, etc., until the correlation did not prove significant any more. This indicates the first year in which increases or decreases became significant.

2.3. Results

2.3.1 Number of diagnostic submissions

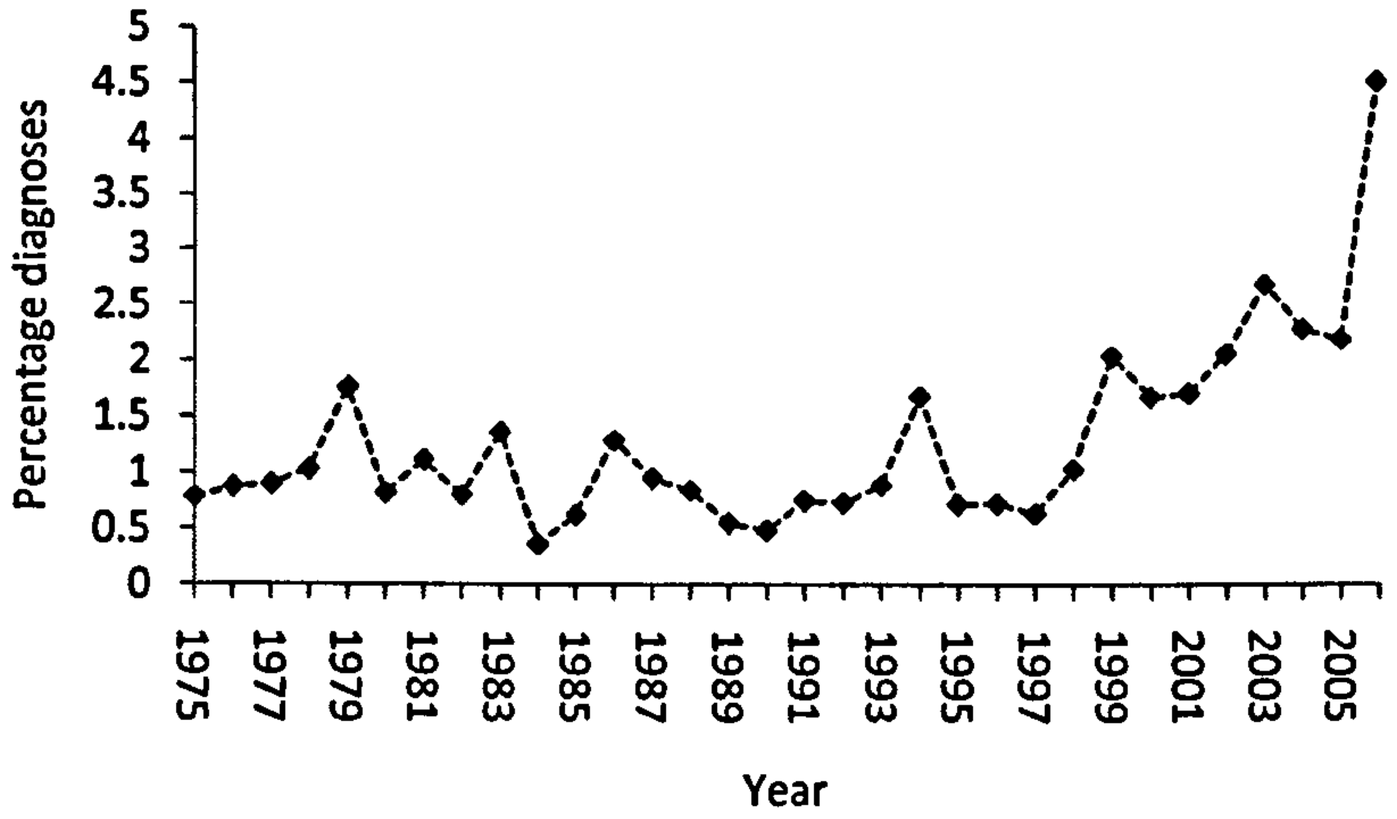
The total number of submissions from sheep to the VIDA laboratories showed a steady increase during the 1980s, followed by a steady decline. Between 1990 and 2006 the total number of submissions was negatively correlated with time (Spearman $r_s = -0.79$, $p < 0.001$). However, since the number of sheep in Great Britain also declined during this period, the number of diagnostic submissions per head of sheep remained relatively constant from year to year ($r_s = 0.06$, $p = 0.85$). Regional submissions to the Southwest, Wales, the

Midlands, the North of England and Scotland all showed a similar decline during 1990-2006 ($r_s \leq -0.63$, $p \leq 0.005$, slopes of linear regressions not significantly different). There was no correlation between number of submissions per year and the price of lamb (pence per kg dressed lamb carcass weight). The dataset analysed represents cases of clinical disease: parasitological monitoring and tests of anthelmintic efficacy are entered separately, and increased uptake of these tests would not affect the total number of submissions or the proportion ascribed to PGE. The total number of tests available at VLA / SAC laboratories has increased rather than decreased during the period studied, and so any increase in the proportion of submissions diagnosed as PGE cannot be ascribed to reduced overall test availability.

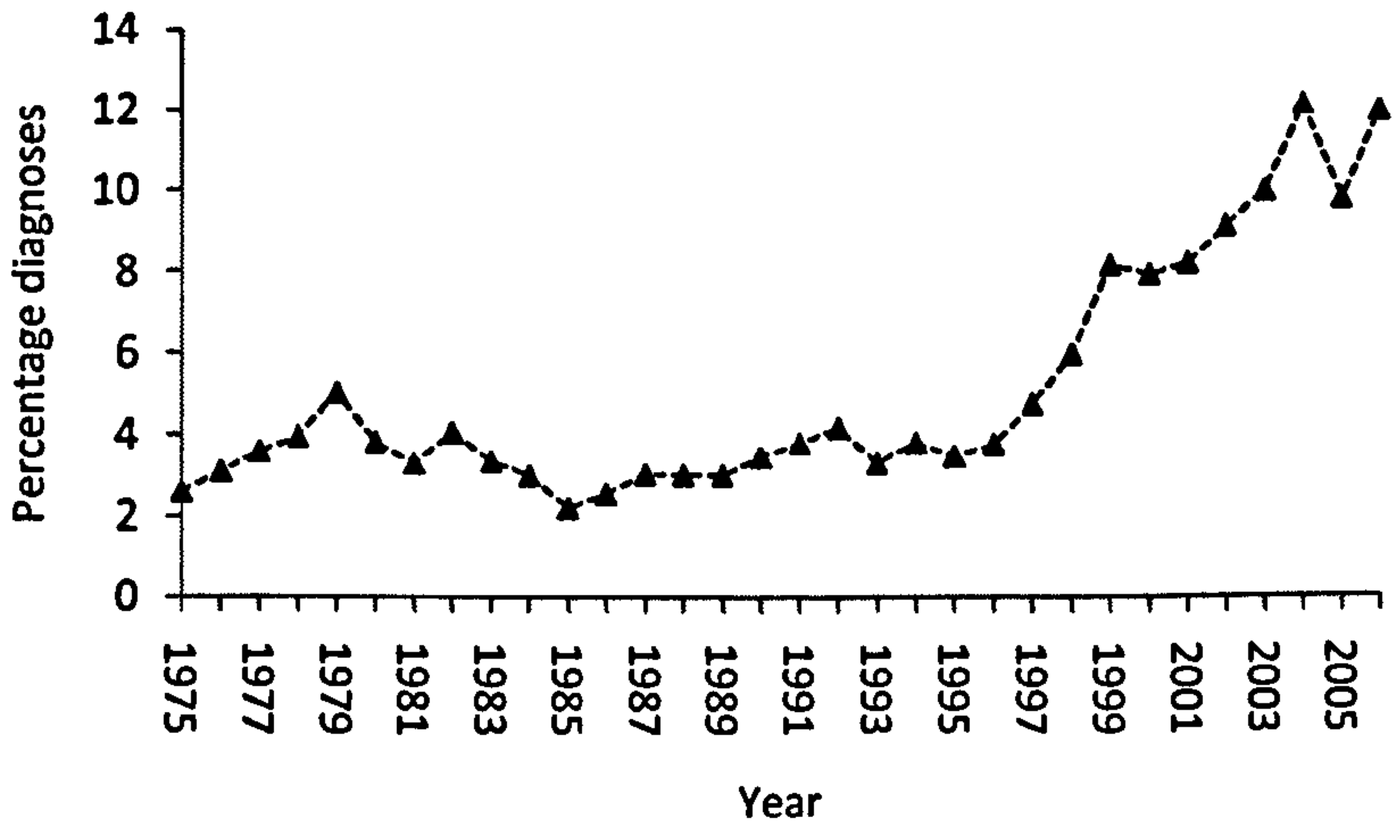
2. 3.2 Average UK trends

The average proportion of total diagnosable submissions attributed to PGE from 1975-2006 is given in fig. 2.1. From the late 1990s onwards, all three categories of PGE appear to show a marked and persistent rise, which is significant over the whole period (table 2.3). For all three categories, the differences between the five year blocks is also significant (table 2.3). *Post hoc* tests show that for nematodirosis only the rise in the last five years (2002-06) is significant ($p \leq 0.011$). For NOS the proportion of diagnoses in 1997-2001 is significantly higher than in all previous years ($p < 0.001$) and the proportion of diagnoses in 2002-06 is again significantly higher than in 1997-2001 ($p < 0.001$). For haemonchosis, a category showing much more variability between years, there are significant differences only between the years 1992-96 and 2002-06 ($p = 0.010$).

Nematodirosis



NOS



Haemonchosis

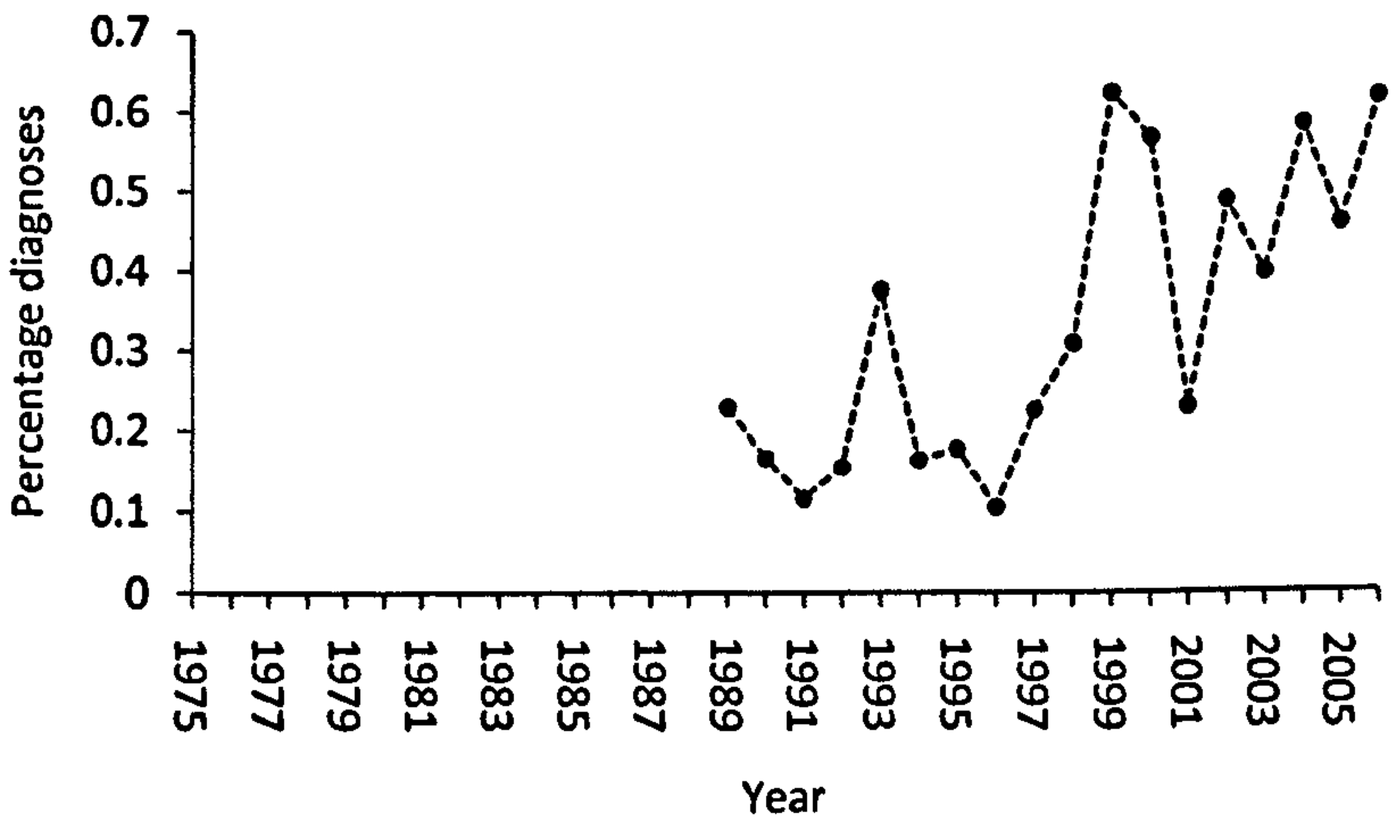


Fig. 2.1 Great Britain averages of diagnoses of Nematodirosis, nematodosis- Not Otherwise Specified (NOS), 1975-2006, and Haemonchosis, 1989-2006, expressed as percentages of diagnosable submissions. Diagnoses in all three classes of nematodes show a marked rise, apparently starting around 1998.

Category	Spearman r_s	p	ANOVA	p
Nematodirosis	0.450	0.005	$F_{5,29} = 8.82$	< 0.001
NOS	0.785	< 0.001	$F_{5,29} = 49.25$	< 0.001
Haemonchosis	0.735	0.001	$F_{2,14} = 6.46$	0.012

Table 2.3 The Spearman correlation coefficient and corresponding p-values for the correlation between diagnosis percentages and time for GB data, 1975-2006, and *F* and p-values of the ANOVA's carried out on 5-year blocks.

2.3.3 Regional trends

Temporal correlations in the proportions of submissions attributed to each category of nematode are given for each region in table 2.4. There are marked differences between the regions and between the nematode categories. The overall national increase in diagnoses of clinical disease due to *Nematodirus* spp. is explained by a highly significant rise in Scotland, which represents approximately one third of the submissions, while increases have not been recorded in other parts of Great Britain. *Teladorsagia* and *Trichostrongylus* species (NOS), however, have caused significantly more disease all over Great Britain during this period. Haemonchosis seems to have been increasingly diagnosed in Scotland, the North and the Midlands, but not in the Southwest or Wales.

Category/region	r_s	p
Nematodirosis		
South West	-0.144	0.215
Midlands	0.101	0.292
Wales	0.251	0.083
North	0.259	0.076
Scotland	0.676	< 0.001
NOS		
South West	0.760	< 0.001
Midlands	0.804	< 0.001
Wales	0.841	< 0.001
North	0.596	< 0.001
Scotland	0.363	0.02
Haemonchosis		
South West	0.321	0.097
Midlands	0.738	< 0.001
Wales	0.073	0.386
North	0.699	0.001
Scotland	0.688	0.001

Table 2.4 The Spearman correlation coefficient and the probability of a type 1 error for the correlation between diagnosis percentages and time, for the different regions, 1975-2006.

2.3.4 Regional differences in rates of diagnosis

Nematodirosis

Differences between regions were highly significant (table 2.5). Using *post hoc* tests, the proportion of diagnoses attributed to nematodirosis did not differ significantly between the

regions Southwest, Wales and Midlands ($U \geq 446$, $p \geq 0.376$, $z \leq -0.886$). Similarly, between the North of England and Scotland differences were not statistically significant ($U = 508$, $p = 0.957$, $z = -0.054$). However, in the two northern regions combined, proportional diagnoses were significantly higher than in the three more southern regions ($U \leq 365$, $p \leq 0.01$, $z \geq -1.98$). Diagnoses showed a significant positive trend from south to north and the maximum positive z value was achieved in the sequence south-west, midlands, Wales, north, Scotland.

NOS

Again, significant differences were found between the regions (table 2.5). The proportion of diagnoses attributed to the NOS group of nematodes did not differ significantly between Scotland and Wales ($U = 491$, $p = 0.385$, $z = -0.282$) but all other regions differed significantly ($U \leq 357$, $p \leq 0.01$, $z \geq -2.081$). A positive trend was seen from north to south, the significance of the Jonckheere-Terpstra test being maximised in the sequence north, Scotland, Wales, midlands, southwest.

Category	*H*	p	↑CL	↓CL	*J*	*z*	*r*
Nematodirosis	14.55	0.004	0.005	0.002	6318	3.61	0.29
NOS	22.88	<0.001	<0.001	<0.001	2614	7.55	0.60
Haemonchosis	27.57	<0.001	<0.001	<0.001	946	4.80	0.51

Table 2.5 H statistics and p values, and their upper and lower 99% confidence intervals, of the Kruskal-Wallis tests, and maximised J statistics, z values and effect sizes of the Jonckheere-Terpsta tests performed on regional data. NB: N = 150 for nematodirosis and NOS and N = 75 for haemonchosis.

Haemonchosis

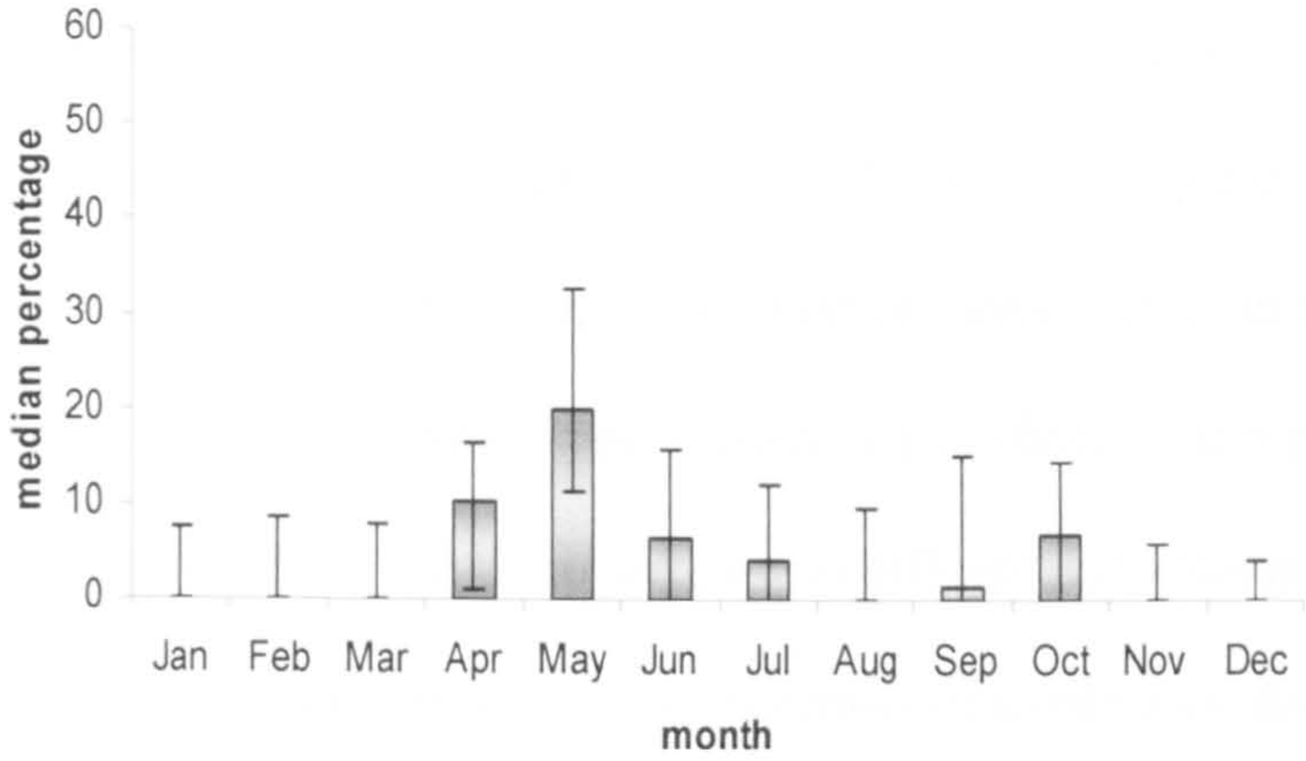
Although significant differences were found between the regions (table 2.5) and the percentage of diagnoses was higher in the southern regions, a clear north-south pattern was not present. Positive z values in the Jonckheere-Terpstra test were maximised in the sequence Midlands, Southwest, North, Wales, Scotland. A sharp divide was apparent between the first three regions and the latter two. Similar levels were found in the Midlands, Southwest and North ($U \geq 140$, $p \geq 0.240$, $z \leq -0.240$) and in Wales and Scotland ($U = 157$, $p = 0.431$, $z = -0.174$), but the difference between the north of England and Wales was highly significant ($U = 78$, $p = 0.003$, $z = -2.675$).

2.3.5 Seasonal patterns

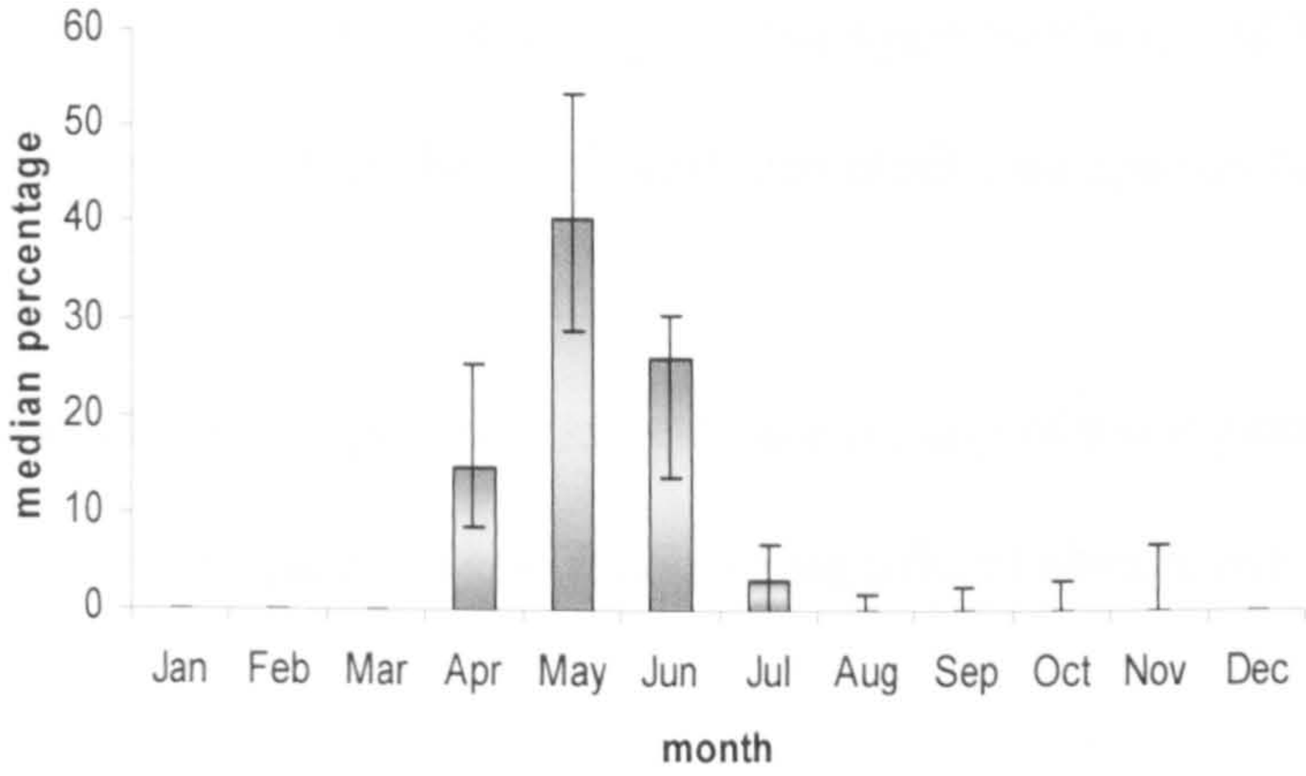
Nematodirosis

It appears that in Wales, and even more so in Scotland, nematodirosis shows the classical highly seasonal, peaked appearance (fig. 2.2). In Wales reported disease increases dramatically from April onwards and peaks in May. Analysis of variance confirmed significant differences between the months of the year ($F_{11, 359} = 55.41$, $p < 0.001$) and identified April, May and June as the peak months (Tukey's $p < 0.001$). In Scotland a similar peak starts approximately one month later and disease incidence peaks in June. Again, differences between months were highly significant ($F_{11, 359} = 109.18$, $p < 0.001$) while the cluster of months May, June and July had a higher percentage of cases than all other months

Southwest



Wales



Scotland

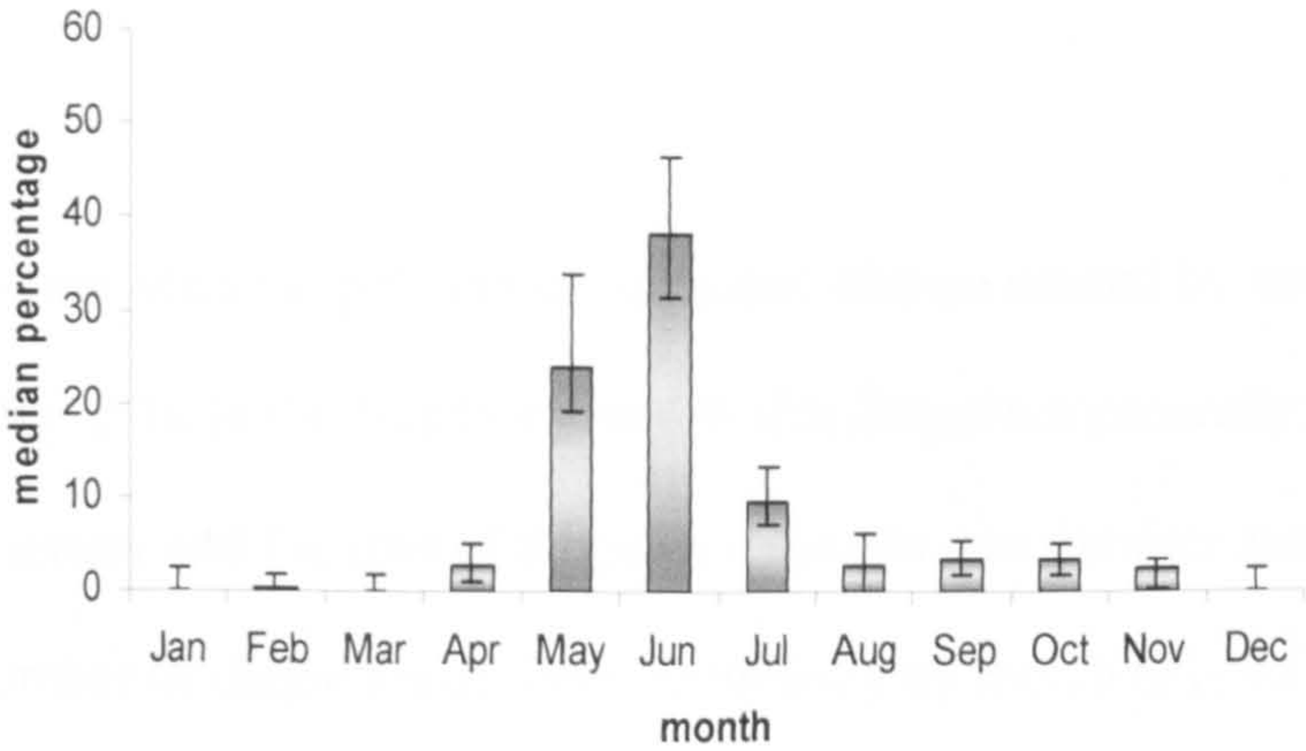


Fig. 2.2 Nematodirosis: Distribution of the diagnoses over the months of the year, as a percentage of the total number of diagnoses for each year, 1977-2006, for the Southwest, Wales and Scotland. Error bars represent the lower (25%) and upper (75%) quartile ranges

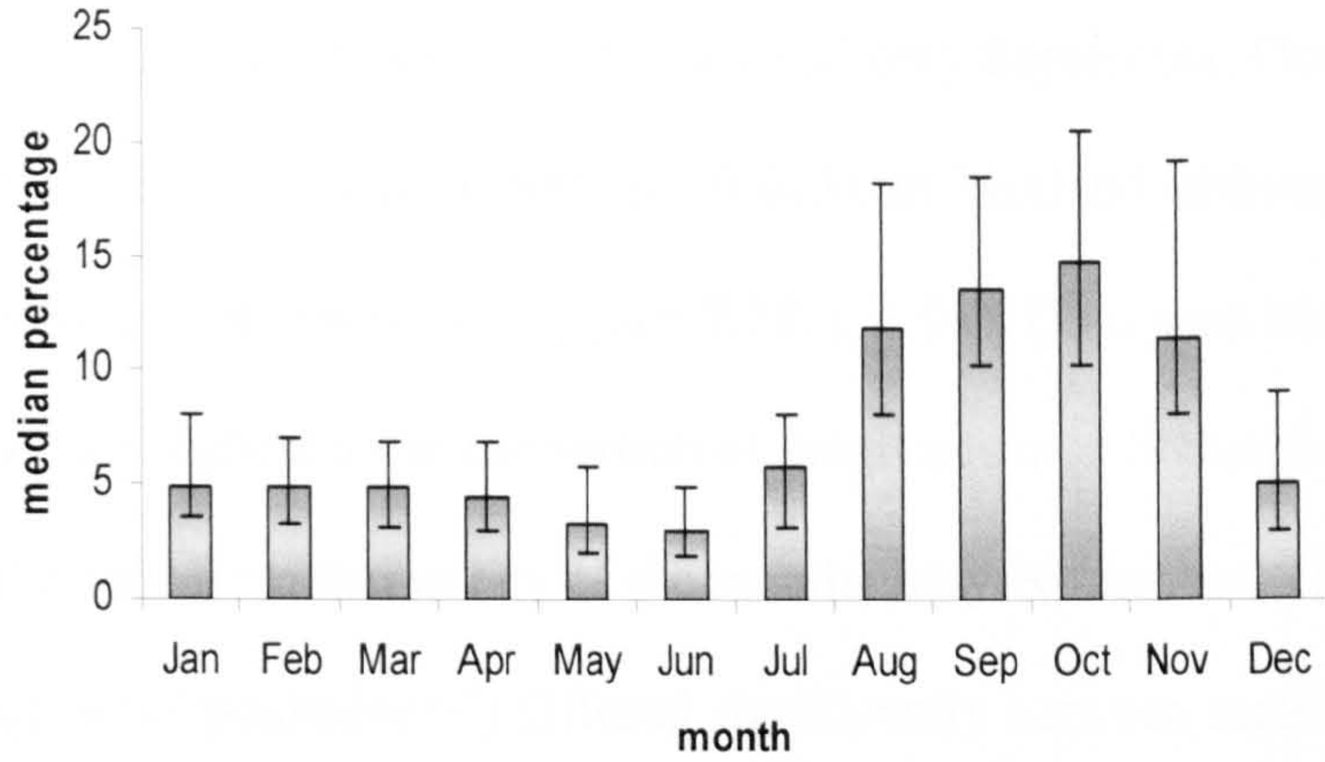
($p < 0.001$). In the Southwest the magnitude of the peak is much smaller. While differences between months were still significant overall ($F_{11, 359} = 7.89$, $p < 0.001$), only in May was the percentage of diagnoses higher than in other months ($p \leq 0.034$). There were significant differences between regions in the temporal concentration of *Nematodirus* in the peak months ($F_{2, 89} = 26.64$, $p < 0.001$). The Southwest showed a significantly less peaked pattern than Scotland and Wales ($p < 0.001$) but there were no significant differences between Scotland and Wales ($p = 0.060$). The importance of autumn nematodirosis also differed between regions ($F_{2, 89} = 8.10$, $p = 0.001$). Autumn percentages were significantly higher in the South-west ($p \leq 0.030$) than in both other regions but again Scotland and Wales were not different to each other ($p = 0.356$). In the Southwest, nematodirosis appears to be much more of an all-year-round disease.

There was no correlation between 'peakedness' and year for any of the regions ($r \leq 0.135$, $p \geq 0.475$), and the five-year comparisons also found no significant change over time ($F_{5, 29} \leq 0.81$, $p \geq 0.555$).

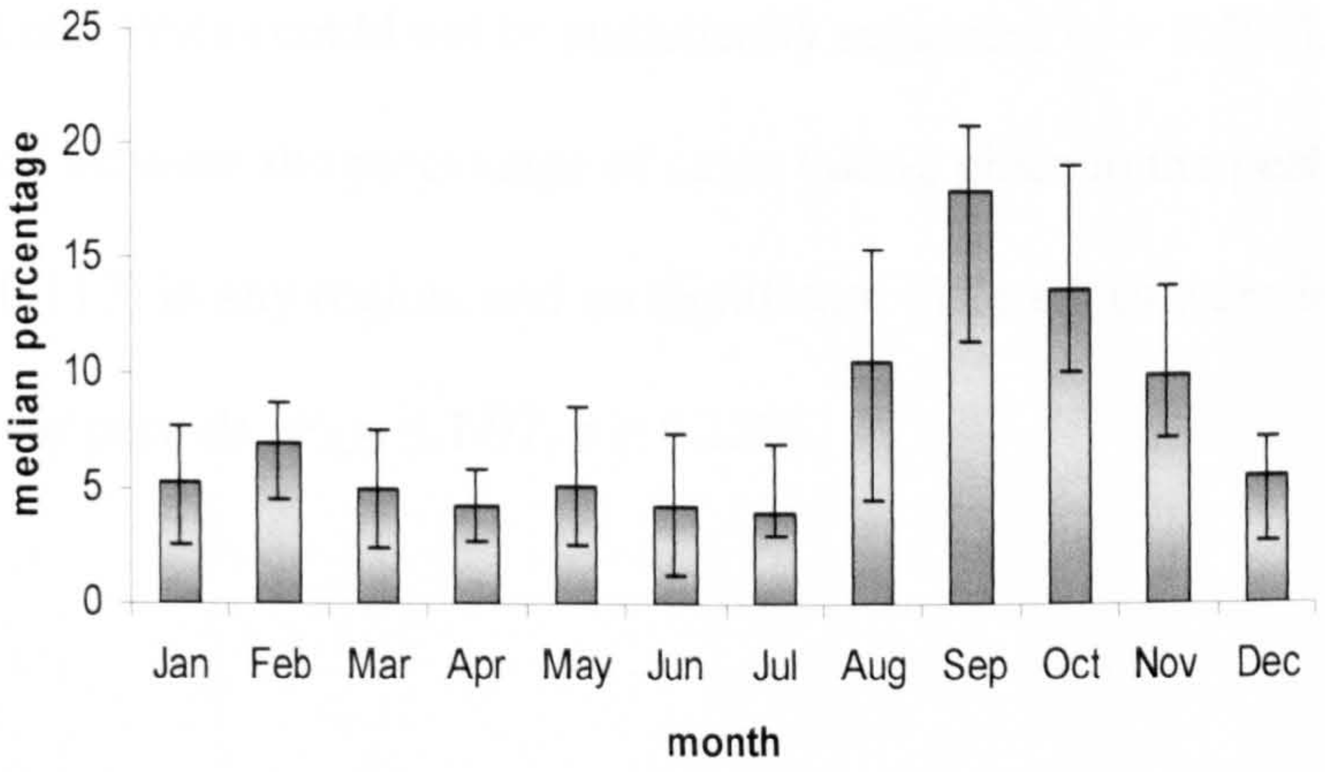
NOS

There are rather different seasonal patterns in diagnosed disease caused by nematode species in category 'NOS' (fig. 2.3). In the Southwest and Wales diagnoses generally decline gradually between January and the start of the peak season in late summer and autumn. In the Southwest the number of diagnoses differs significantly by month ($F_{11, 359} = 22.08$, $p < 0.001$). The months August, September, October and November show a significantly higher percentage of cases than all other months ($p < 0.001$) but do not differ significantly

Southwest



Wales



Scotland

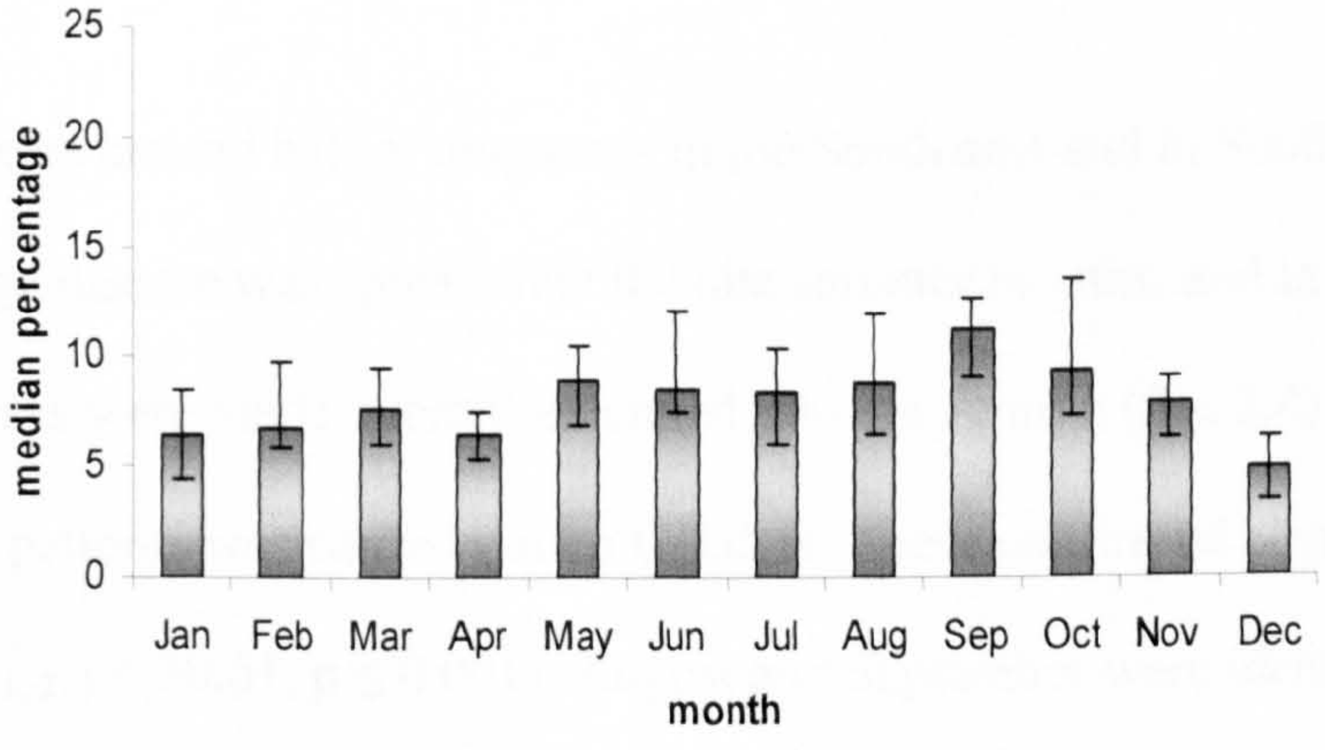


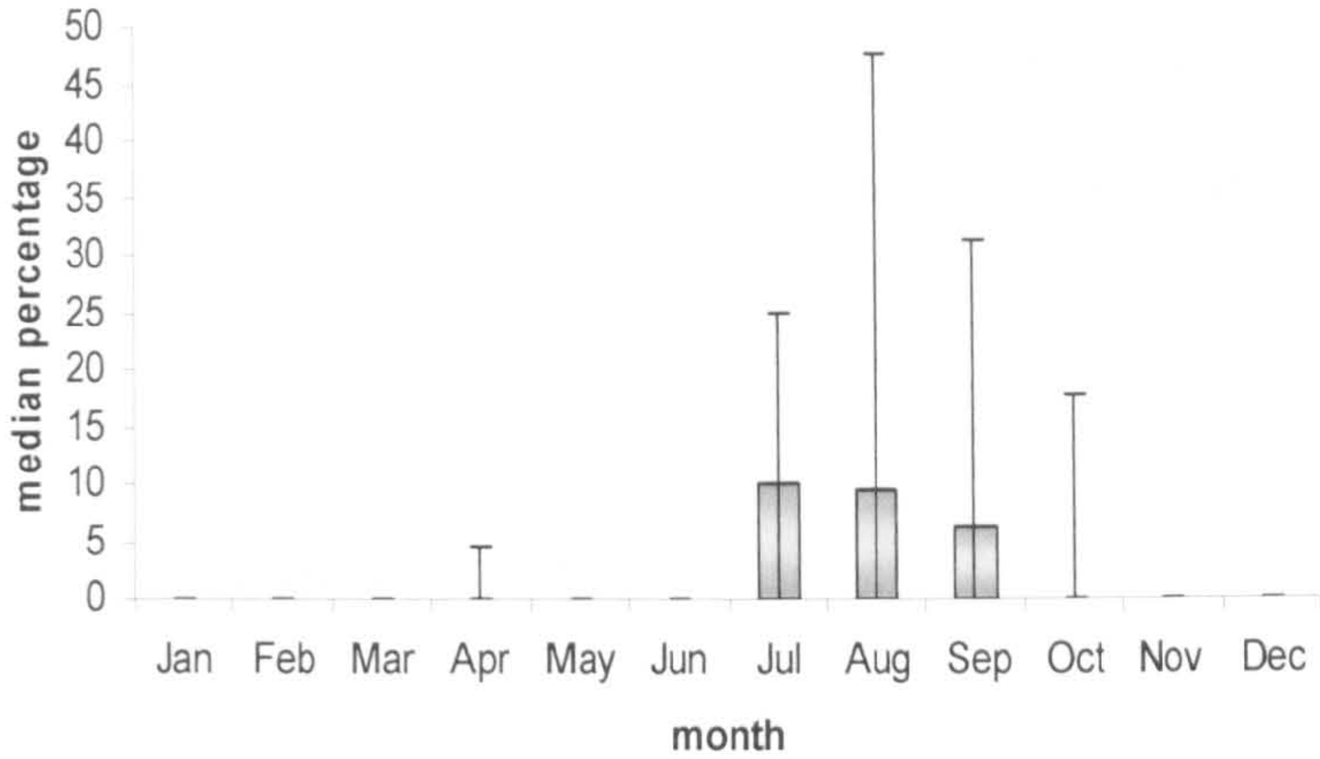
Fig.2.3 Nematodosis-NOS: Distribution of the diagnoses over the months of the year, as a percentage of the total number of diagnoses for each year, 1977-2006, for the Southwest, Wales and Scotland. Error bars represent the lower (25%) and upper (75%) quartile ranges.

from each other ($p \geq 0.597$). In Wales diagnoses also vary by month ($F_{11, 359} = 18.74$, $p < 0.001$). However, the peak season is less well defined and only September, October and November have more cases than other months ($p \leq 0.023$). In Scotland, although significant differences across months were detected ($F_{11, 359} = 7.78$, $p < 0.001$) no peak disease season was discernible. Thus, the highest mean proportion of diagnoses is in September but this does not differ significantly from that in any of the months May-November. The temporal concentration of diagnoses ('peakedness') differed significantly between regions ($F_{2, 89} = 22.91$, $p < 0.001$). Scotland showed a less peaked pattern than both other regions ($p < 0.001$), while the Southwest and Wales could not be statistically separated ($p = 0.590$). There was no significant correlation between the percentage of cases taking place in the peak seasons and year ($r \leq 0.297$, $p \geq 0.112$) in any region, and no significant differences were found between any of the six five-year periods ($F_{5,29} \leq 1.97$, $p \geq 0.120$).

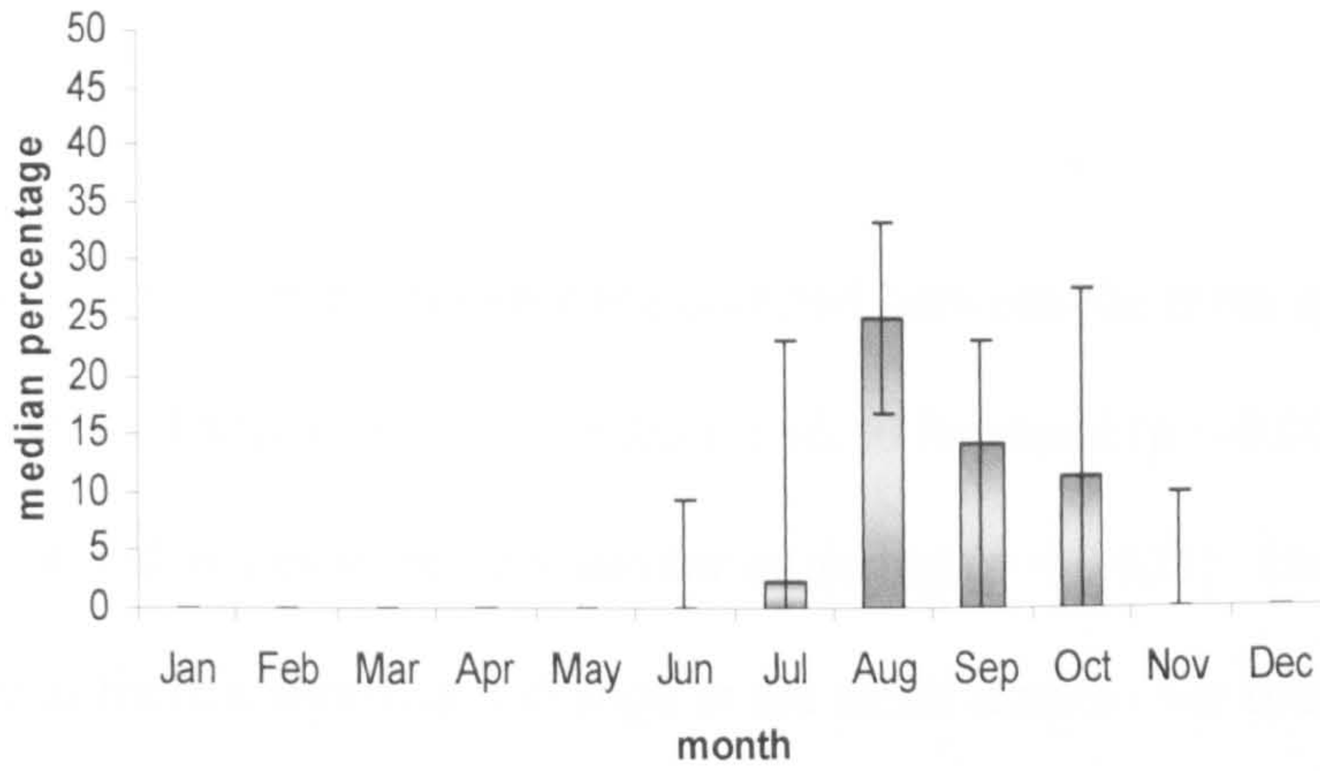
Haemonchosis

Although in some years around half of diagnoses in the Southwest and in Scotland occurred in August, on average disease was spread over the late summer months, and in the Midlands (where more diagnoses were made overall) extended into the autumn (fig. 2.4). Detailed analysis of seasonal patterns was carried out on GB data. These confirmed clear differences between months ($F_{11, 215} = 30.61$, $p \leq 0.001$). August and September were identified as the peak months, showing significantly more diagnoses than all others ($p \leq 0.001$).

Southwest



Midlands



Scotland

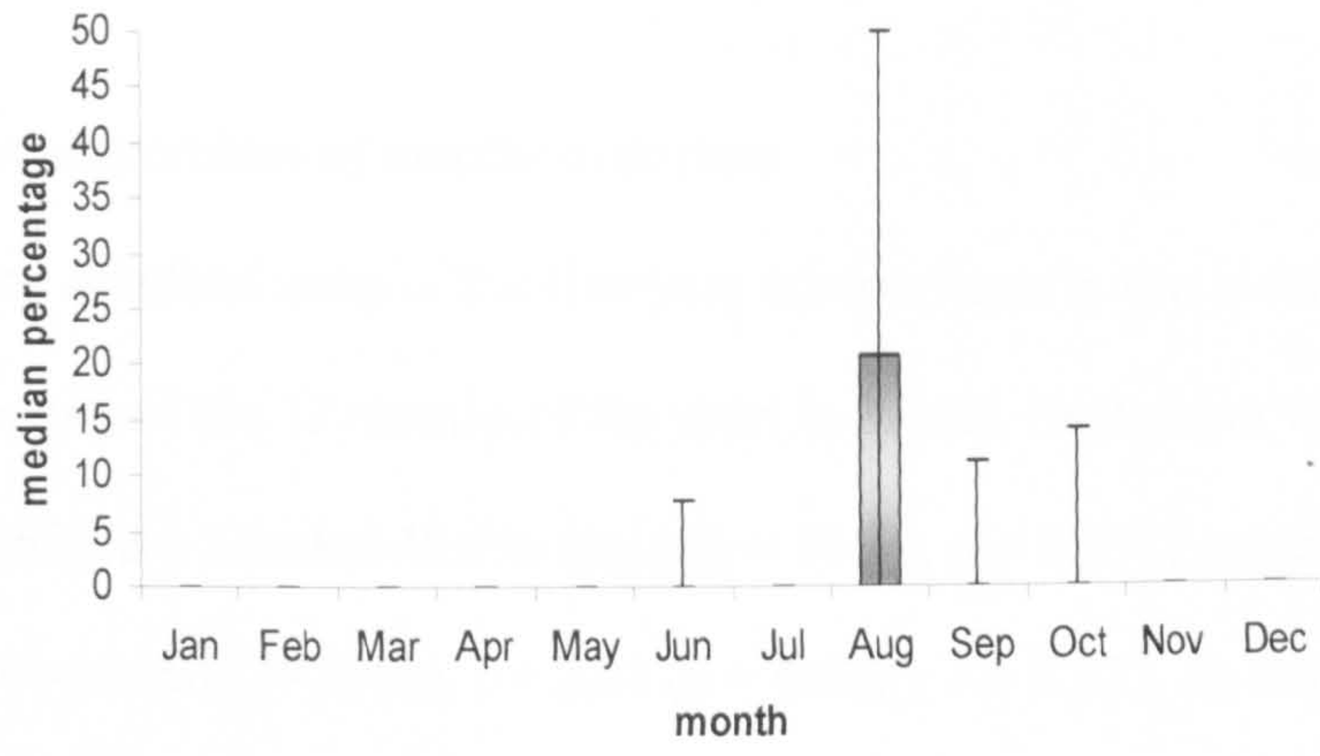


Fig. 2.4 Haemonchosis: Distribution of the diagnoses over the months of the year, as a percentage of the total number of diagnoses for each year, 1989-2006, for the Southwest, the Midlands and Scotland. Error bars represent the lower (25%) and upper (75%) quartile ranges.

There was a significant decrease in the proportion of cases taking place during the peak months over the past 30 years (r = -0.604, p = 0.008). The existence of significant differences in 'peakedness' between three consecutive clusters of six years ($F_{2, 17}$ = 4.34, p = 0.032) supports this finding.

2.3.6 Changes in seasonal patterns 1977-2006

Nematodirosis

a. Peak shifts

Significant differences in mean peak month were detected between the three selected regions ($F_{2, 86} = 14.15, p < 0.001$). These were due to a later peak in Scotland (p < 0.001), while the peaks in the Southwest and Wales were very similar in timing (p = 0.623). However, for none of the regions was there a significant change in the mean month over time ($r_s \leq 0.18$, p $\geq$ 0.359).

b. Changes in relative importance of months over time

For the Southwest and Scotland none of the five-year comparisons or the trend tests yielded significant results for any of the 12 months of the year. In Wales, September was the only month in which a significant Kruskal-Wallis test (H_4 = 10.69, p = 0.037) was accompanied by a significant positive trend (J= 236.0, z = 2.01 (p = 0.022), r = 0.37). As September percentages over 1997-2001 were not significantly higher than in any of the four previous time blocks (U= 12.50, p = 1), and the 2002-2006 percentages were not significantly higher

than in 1997-2001 ($U = 9.00$, p = 0.459), this trend is not the result of recent sharp rises but rather continuous increase over the past 25 years. A single significant Jonkheere-Terpstra test in December ($J = 229.0$, $z = 2.33$ (p = 0.001), $r = 0.43$) indicated that cases started to occur in this month from 1999 onwards.

NOS

a. Peak shifts

As no peak period could be identified for Scotland, this analysis was conducted only for Wales and the Southwest. No difference was found in mean peak month between regions ($F_{1, 59} = 0.014$, p = 0.908). The mean peak month became significantly later over the years in the Southwest ($r_s = 0.382$, p = 0.037) but not in Wales ($r_s = 0.146$, p = 0.440).

The result for the Southwest was confirmed by a significant Jonckheere-Terpstra test on successive 5-year blocks ($J = 234.0$, $z = 1.69$ (p = 0.046), $r = 0.31$).

b. Changes in relative importance of months over time

A striking number of significant negative Jonckheere-Terpstra trend tests were detected in the months April, May and June. In the Southwest trends were significantly negative in May and June ($J \leq 132$, $z \leq -1.65$ ($p \leq 0.05$), $r \leq -0.29$), in Wales in April ($J = 130.5$, $z = -2.07$ (p = 0.02), $r = -0.38$) and in Scotland in April and June ($J \leq 113$, $z \leq -2.70$ ($p \leq 0.01$), $r \leq -0.49$). This indicates relative declines in spring 'NOS' nematodosis over the period 1977-2006. The probability of the five significant tests appearing in the months April, May and June in these three regions as the result of type I errors is 1.61×10^{-10}.

Haemonchosis

a. Peak shifts

The mean peak month did not change significantly with year ($r_s = 0.199$, p = 0.428).

b. Changes in relative importance of months over time

There were no differences in monthly patterns between the three five-year blocks compared ($H_2 \leq 3.567$, $p \geq 0.165$). Jonckheere-Terpstra tests, however, identified significant positive trends for the months November ($J = 65.5$, $z = 2.05$ (p = 0.020), $r = 0.53$) and December ($J = 54.0$, $z = 1.84$ (p = 0.033), $r = 0.48$), suggesting greater autumn transmission of *Haemonchus contortus*.

2.3.7 Climatic trends

The results of the trend analysis over 1975-2006 are given in table 2.6. The mean annual temperature has increased in the past ten years when compared with 1975-1996. However, this does not constitute a significant trend for all months. It is striking that mean temperatures increased more significantly, and much sooner, in February and the following spring months than later in the year. No clear patterns in rainfall were detected except a significant increase in the amount of rain in April.

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Temp.												
M	3.5	3.6	5.3	7.2	10.2	13.0	15.1	14.9	12.6	9.5	6.2	4.2
T_{10}	+0.8	+1.4	+1.0	+0.8	+1.0	+0.6	+0.3	+0.8	+1.3	+0.6	+0.8	+0.6
r_s	0.30	0.33	0.43	0.46	0.48	0.30	0.19	0.29	0.53	0.31	0.37	0.10
p	0.04	0.03	0.007	0.004	0.003	0.04	0.15	0.06	0.001	0.09	0.02	0.30
year	2005	1998	1999	1999	1999	2006	-	-	2002	-	2003	-
Rainfall												
M	116	80	94	70	65	69	67	82	96	122	115	120
R_{10}	-11	+18	-21	+19	+14	+16	+4	+6	-9	+22	+13	+8
r_s	0.00	0.25	-0.33	0.43	0.19	0.15	0.17	0.07	-0.22	0.26	0.11	0.04
p	0.96	0.17	0.065	0.014	0.31	0.42	0.36	0.69	0.22	0.16	0.55	0.81
year	-	-	-	1999	-	-	-	-	-	-	-	-

Table 2.6 Temperature and rainfall trends during 1975-2006. M = 1975-2006 average mean monthly values; T_{10} and R_{10} = the change in the mean monthly temperature and millimetres of rainfall, in °C and millimetres, respectively, of the past 10 years, when compared to the 22 previous years; r_s and p = the Spearman correlation coefficient and the corresponding p value; 'year' = the year in which the increase first became significant.

2.4 Discussion

Within the limitations of the conditions under which VIDA data are collected, laboratory records of parasitic gastroenteritis in sheep in Great Britain show a clear increase in the number of diagnoses of all three categories of disease over the past 32 years. The timing of this increase differs between groups of nematode species, but appears to have occurred mainly over the past 5-10 years. This coincides with the beginning of grossly detectable changes in climate, notably increases in spring and autumn temperatures. Though it is tempting to link increasing disease records to climate change, the data are descriptive and it

is impossible to exclude alternative hypotheses. These can, in the main, be divided into reporting bias, changing husbandry patterns, and anthelmintic resistance.

Formal PGE case definitions were introduced in 1999 but they tend to exclude diagnoses rather than increase their numbers and would not be responsible for an increase trend in diagnoses. Although the overall rate of submission of sheep samples to VLA diagnostic laboratories has varied significantly over the past ten years, the submission rate per head of sheep has not. The proportion of total diagnoses attributed to PGE is therefore unlikely to be significantly affected by changes in the number of diagnoses of other diseases. The total submission rate furthermore appears to be insensitive to fluctuations in farm revenue as reflected by lamb prices. It is conceivable that the incidence of PGE and farmer motivation to submit samples are both affected by general changes in the sheep production sector such as intensification. However, between 1999 and 2005 the UK national flock decreased in size by approximately 20%, while the total number of hectares of permanent grassland increased by 5% over a similar period (Defra 2007b). At the same time there was a steady increase in the number of holdings on which sheep are kept, to a total of 15%. While the number of registered holdings between 5 and 100 hectares decreased, the number of holdings above 100 hectares and below 5 hectares both increased. Lower sheep numbers, increased availability of grazing and increasing numbers of both very large - often more extensively managed - farms and smallholdings together make it unlikely that intensification of the sheep industry can have contributed to an increase in diagnoses of PGE. The number of smallholders has increased but relatively few government laboratory submissions are received from this sector. Increased awareness of PGE is also an unlikely explanation for increased diagnoses, since there has been strong awareness of this disease complex among

farmers and vets for many decades, and tests for routine flock monitoring and anthelmintic resistance were excluded from the analysis.

Anthelmintic resistance has certainly increased in importance in the UK during the study period, with recent estimates suggesting that up to 80% of sheep farms have nematodes resistant to at least one class of anthelmintic, and several cases of triple-class resistance have been reported (Bartley et al., 2003; Bartley et al., 2006). However, its contribution to increases in recorded clinical disease is debatable. Most farmers become aware of anthelmintic failure through poor performance in controlling subclinical disease, especially lamb growth rates, and react by changing anthelmintic group before incurring losses worthy of pathological investigation. On the vast majority of farms, effective products were still available during the critical period 1990-2006 (Jackson and Coop, 2000; Coles 2002; Coles, 2003). Moreover, failure of anthelmintic prophylaxis cannot explain the altered seasonal patterns in PGE, or sharp increases in disease caused by *Nematodirus battus*, and the cattle lungworm *Dictyocaulus viviparus* (David, 1997; van Dijk, 2004), species in which anthelmintic resistance has not been recorded. Nevertheless, compromised control as a result of anthelmintic resistance could exacerbate problems due to PGE, and it is impossible to exclude this as at least a partial explanation for the patterns described.

The overall increase in the rate of diagnosis of PGE caused by *Nematodirus* spp., in practice mostly *N. battus*, is accounted for entirely by an increase in Scotland, with no significant change in other regions analysed. This parasite has a strongly seasonal pattern and clinical disease is observed mostly in spring. This is consistent with the widely accepted hypothesis that the mass hatch of *N. battus* is tailored towards the availability of young lambs early in

the grazing season (Gibson and Everett, 1981). The spring peak is stronger and occurs later in the year in Scotland than elsewhere, presumably due to the later spring rise in temperature. The parasite appears to be more commonly diagnosed in northern regions where the spring peak is more pronounced: this could be because the concentration of transmission in spring is important to parasite population growth, or simply that concentrated infection of lambs is more likely to produce disease. The analysis also suggests that in the southern parts of the UK, *N. battus* patterns of disease are likely to deviate substantially from the classic spring peak. In south-west England, this peak is less well defined and disease is recorded in all months. This may suggest that the parasite is not always able to complete its hatch in the spring and that hatching subsequently occurs during later windows of opportunity. The timing of these windows is the result of stochastic variation in local climate and this is reflected in the fact that in most months there are usually no outbreaks, and yet there is the potential for an occasional significant number of outbreaks in any month of the year. *N. battus* eggs are generally thought to hatch when, after cold spells in winter, temperatures rise in spring (Christie, 1962; Parkin, 1972; Gibson and Everett, 1981) and therefore it is unclear why the spring hatching process appears to be completed in other regions but not always in the Southwest. Autumn transmission of *Nematodirus* also appears to be more important in the Southwest than in the other regions. This might reflect higher development rates of eggs during the summer, such that a proportion of eggs is embryonated and able to hatch without chilling in autumn, as well as the presence of a larger number of eggs 'left over' from the incomplete spring hatch. The recent increase in autumn disease in Wales could then be the result of higher spring and/or summer temperatures. Geographical differences in hatching behaviour might equally be

explained by local strain variation, although there is no evidence for variation in the vital rates of free-living stages of trichostrongyloid nematodes at similar spatial scales, and no published information on regional strain variation in *Nematodirus*. Perhaps surprisingly, given the pronounced north-south variation in seasonal patterns of disease, the analysis provides no evidence for a temporal shift in the peak of disease, or the sharpness of the spring peak, over the years in any region. Such changes might be expected if the degree of winter chilling of eggs or the months in which the climate allows hatching have changed in recent decades. Moreover, increased incidence of nematodirosis in Scotland cannot easily be explained from our current published knowledge of the epidemiology of *Nematodirus* spp. In practical terms, it should be noted that the less predictable timing of disease in southern regions should encourage caution in the use of soil-temperature indices to identify years of high and low risk for infection of spring lambs (Thomas, 1991).

Recorded disease caused by nematodes in the 'NOS' category, mostly represented by *Teladorsagia circumcincta* and *Trichostrongylus spp.*, increased in all regions in the past 10 years. Overall rates of diagnosis are higher in the southern regions, which also show more pronounced clustering of outbreaks in the late summer months. This is to be expected from the well described population dynamics of these species (Gibson and Everett, 1967; Beveridge *et al.* 1989), which are characterised by accumulation of infective stages from successive generations of adult parasites and accelerated rates of development of eggs and larvae at higher temperatures, leading to higher parasite abundance and increased risk of disease from midsummer onwards (Armour, 1986). Since sheep in the North are unlikely to spend more time at pasture than those in the South, the more even year-round distribution of

cases in northern regions could be due to increased larval survival at cooler temperatures. Sheep turned out onto pasture contaminated the previous summer and autumn would then be more likely to ingest large numbers of surviving larvae, and disease could occur at any time of year. The pattern of regions in which the parasites are most and least successful is exactly the opposite of that for *Nematodirus*, with the largest relative number of cases being diagnosed in the Southwest. It thus appears that within this group of parasites, as with *Nematodirus*, the most successful transmission pattern is a more 'peaked' one. However, there are clear indications that in the Southwest, significantly more cases have started to occur later in the year. Success may therefore rather be related to an extension of the number of months with a high probability of successful transmission, i.e. the width of the peak plateau. This extension only seems to be occurring into the late autumn and winter months as, across regions, there was a tendency towards fewer cases in the spring and early summer. As no other plausible explanation seems available, the negative trends for cases in these months, detected in several regions, and the occurrence of more cases later in the year in the Southwest, may be the strongest evidence yet that global warming is already having significant effects on the epidemiology of these parasites. These patterns can be explained in relation to the observed increases in temperature. Thus, mean monthly UK temperatures in the coldest months of the year lie very close to the minimum development threshold of the 'NOS' group of parasites, and windows of opportunity for development may therefore already occur during these months. Increased temperatures will tend to increase the proportion of larvae developing to the L3 stage and migrating onto grass in winter, while also decreasing larval survival. Availability of L3 to susceptible lambs would consequently be lower early in spring but this would be compensated for by more rapid build up of pasture

contamination due to shorter generation times later in the year, leading to increased disease risk later in the summer, and extension of the transmission period later into the autumn. It is also possible that decreased exposure to infection early in the year delays acquisition of immunity and enables higher individual burdens to build up later on. It is striking that in all regions PGE diagnoses decreased in the very months in which significant warming first started to occur.

The analysis of haemonchosis was limited by small regional sample size but confidence in the results is enhanced by the emergence of patterns consistent both across tests within the category, and with the findings in the category 'NOS'. In the Midlands, where it is most successful, *Haemonchus* has been able to expand its transmission window the most consistently into the autumn. This trend is also reflected in the decrease in the 'peakedness' of the *Haemonchus* season in the UK, and the positive trends for disease outbreaks in November and December. Overall increases in diagnosed disease were only significant for more northern regions, suggesting that in recent years the parasite has especially benefited in the regions where thermal energy was most limiting. The reasons behind the high incidence in the Midlands are unclear.

Although this study is essentially descriptive and cannot prove that that the observed patterns are the result of climate change, this is the most likely explanation. Increases in UK average temperatures coincide closely in time with observed increases in PGE and explain patterns not accounted for by alternative hypotheses. It is not surprising that climate change should produce changes in epidemiology, especially for diseases caused by parasites whose

development outside the definitive host is sensitive to temperature and humidity (Poulin, 2006). Predictions of far-reaching effects of climate change on disease dynamics in natural systems (Harvell *et al.* 2002) have been borne out by detailed studies of trematode parasites in marine ecosystems (Poulin and Mouritsen, 2006), nematodes of ungulates in the arctic (Kutz *et al.* 2005; Jenkins *et al.* 2006b), and a range of vector-borne parasites worldwide (Harrus and Baneth, 2005). However, this is the first documented evidence of systematic changes in the epidemiology of trichostrongyloid nematodes of grazing livestock, and of GB endemic disease in general, that may be ascribed to increased regional mean temperatures in the past decade.

Detection of changes in the epidemiology of PGE is important so that advisors and veterinarians can consider altering recommendations concerning control. These data highlight the importance of conducting this surveillance at an appropriate spatial scale, since changes in the overall incidence of disease and its seasonal occurrence can vary from region to region, and too general an analysis could miss important local patterns. It is of great value to collect data using protocols that are standardised between laboratories and over time, so that trends in disease epidemiology can be captured without excessive bias. The lack of published evidence for changes in the epidemiology of parasites of veterinary importance with ongoing climate change, in spite of overwhelming evidence for the importance of climate in driving transmission patterns, might be explained by the rarity of such databases. The current analysis should therefore serve not only as a baseline for future studies, but also a source of testable hypotheses regarding the effect of climate change on the behaviour of the parasites concerned.

Assuming that mean temperatures continue to increase in the UK, further changes in the epidemiology of PGE in sheep can be expected. Should the future climate of the northern parts of Great Britain come to resemble the current climate of the south, *Nematodirus battus* hatching will presumably become more erratic, leading to decreases in the number of disease outbreaks but also a decrease in their predictability. This might compromise control efforts that target preventative measures such as pasture rotation and chemoprophylaxis to the expected peak time of infection (Thomas, 1991). Other species groups such as *Teladorsagia circumcincta* and *Trichostrogylus* spp., on the other hand, might show more concentrated increases in abundance in late summer, extending into autumn, with increased risk of disease in this period. This is assuming that moisture will not become limiting for development of the free-living stages. Although average summer rainfall shows no consistent pattern in recent years, more common droughts would be expected to restrict transmission to times of higher rainfall such as early autumn. Increased summer temperatures would also be expected to reduce larval survival on the pasture. This may be especially important for *Haemonchus contortus* (Besier and Dunsmore, 1993) and could provide opportunities for control through grazing rotation that are currently undermined by high survival rates of larvae on pasture in temperate areas (Eysker *et al.* 2005*a*). However, the present analysis provides no evidence that increasingly difficult conditions for the free-living stages in summer are having any effect on observed patterns of disease. This is in agreement with the observed resilience of *Haemonchus contortus* to dry summer conditions in the Netherlands (Eysker *et al.* 2005*b*). Moreover, changes in climate could drive parasite adaptation, so predictions based on vital rates estimated from current field strains could become outmoded in future. Parasite strategies would be expected to evolve to enhance persistence in changed environments, for

example decreased larval survival on pasture in summer or in winter could select for prolonged survival in the host, e.g. through hypobiosis (Waller *et al.* 2004). At the same time, decline in the typical proportion of the total parasite population represented by the free-living stages could also increase selection for anthelmintic resistance through reduced refugia (van Wyk, 2001; Papadopoulos *et al.* 2001). Changes in the timing of host infection could affect the acquisition of immunity, with potentially complex effects on disease incidence. Any future effect of climate change on the larval dynamics of these parasites will also be modified by host grazing patterns, which could change concurrently as temperature shifts alter patterns of grass growth. Predicting the effects of climate change on the epidemiology of PGE is therefore complicated and should take into account climatic stochasticity as well as changes in average temperature and rainfall (Morgan *et al.* 2004), and also changes in host and parasite behaviour. In any case, the direct dependence of transmission on temperature-dependent vital processes in the free-living stages make trichostrongyloid nematodes very likely early biological indicators of climate change.

2.5 Conclusions

In conclusion, the results presented show that, in Great Britain, overall diagnosis of PGE in sheep has increased at an alarming rate over the past 5-10 years. This cannot be explained by obvious sources of bias, but can be explained by expected effects of changing temperatures on vital rates of the free-living stages of the relevant parasite species. There are clear geographical differences in disease patterns, suggesting that a regional approach to worm control, and epidemiological surveillance, is desirable. In the future, different parasite

species in the PGE complex are likely to respond in different ways to ongoing climate change. Seasonal rates of diagnosis suggest that, in line with increases in temperature, fewer larvae of *Teladorsagia* and *Trichostrongylus* species survive the winter and spring at pasture, while the windows of transmission of these species, and of *Haemonchus contortus*, have extended into the autumn. Since the effect of climate change on parasitic species depends on a complex network of factors, an integrated approach to control should consider the effect of within- and between-year variation in climate on vital rates of the free living stages alongside host grazing practices and immunity.

Comparison of disease patterns across climatic zones within Great Britain may assist in the formulation of hypotheses on parasite behaviour at pasture and give an insight into future epidemiology.

Chapter 3- Temperature and parasite abundance

3.1 Introduction

Anderson and May (1991) first defined the basic reproduction ratio (R_0) for macro parasites as "the average number of female offspring produced through the life-span of a mature parasite that themselves survive to maturity in the absence of density-dependent constraints to population growth". Following on from this Heesterbeek and Roberts (1995) introduced the basic reproduction number (Q_0), the expected number of adult progeny arising from a single adult female introduced into a previously unexposed host population on clean pasture. Kao *et al.* (2000) successfully parameterized this model for several species of gastrointestinal nematodes of sheep. Working on a time scale of one year, with mean annual values, they showed that the model could be used as a measure of predicted success of a certain species in a certain country, and to compare between countries located at different latitudes. In the previous chapter a range of apparent alterations in parasite epidemiology was identified. Kao *et al.* (2000) stated that "simple analytical models can be used to elucidate general principles but are less useful in predicting the results for specific situations". This chapter will explore whether a simple Q_0 model approach, applied to different temporal and spatial scales, includes enough complexity to explore whether the observed UK patterns can be ascribed to changes in temperature alone.

The Q_0 model predicts the progeny of a worm present in a non-immune host which is put out to clean pasture (i.e. pasture that has not been grazed by infected sheep for a period of time sufficiently long enough for all infective larvae to have died). However, this is not a realistic

scenario for intensively farmed UK sheep. Depending on the parasite species, at turnout, lambs may encounter L3 which have developed in the previous calendar year and thus infection rates at this time are also a function of both development rates in the previous autumn and survival over the winter period. As chapter 2 hypothesized that increased temperatures may impair over-winter larval survival, lowering spring disease incidence, the model used in this chapter will be explicitly extended to the presence of autumn-developed larvae. A second important adaptation on the Q_0 approach used by Kao *et al.* (2000) is the use of daily time steps, in which larval development and death rates are made a function of temperature, instead of a generic function for seasonality.

Specific questions to be addressed by the model are:

1) Can changes in daily temperature over the past 30 years alone explain the observed changes in disease patterns, and differences in regional patterns, or do more climatic variables (i.e. patterns of rainfall, hours of sunlight etc.) have to be included into a model predicting parasitic success?

2) In the UK, is parasite abundance mainly driven by temperature constraints on larval development, or by larval death rates, or are the two of equal importance? In which direction will this be altered by climate change?

3) What is the potential contribution of the over-wintering of infective larvae outside of the host to the success of three different parasitic nematodes?

4) Is it appropriate to model parasite development and death on mean (daily) temperatures, or would models necessarily have to include the range of temperatures encountered over that day?

3. 2 The model

The model explores recruitment into the adult parasite population through the availability of infective larvae, and losses of adult parasites as a result of ageing, and has two state variables: the mean number of adult parasites per host *A*, and the pasture density of infective nematode larvae *L*. It projects and adds the success rate of two adult worms of a certain species, expressed as the adult progeny per adult female: 1) one present in a non-immune host at the time of turnout onto pasture in a year under study, assuming that the temperature conditions will stay the same and sheep will stay on that plot of land long enough for development of L3, and ingestion of these, to occur, and 2) a worm present in a host in the previous September, assuming its eggs, if they develop, reach the L3 stage in the last two weeks of September, are present as L3 on October 1st, and spend the subsequent winter at pasture. Surviving L3 are then encountered by the lamb put on the pasture on day *t* of the following year.

On any given day the projected, future, change in the number of adult parasites in the population equals the temperature-dependent recruitment resulting from eggs shed on that day plus the recruitment as the result of ingested autumn-developed larvae, if present, minus the death rate of adult worms. The change in the number of L3 available for ingestion by one host equals the loss of larvae due to temperature-dependent death plus the loss of larvae as the result of ingestion by other hosts. The dynamics of the system are described by the differential equations (a definition of the model parameters, and the assumptions attached to them, is given in table 3.1)

$$dL/dt = P_{d(T)} \lambda H A + P_{s(T)} P_{w(T)} \lambda H A - (\mu_{1(T)} + \beta H) L \qquad \text{Equation 3.1}$$

$$dA/dt = q \beta L - \mu_2 A . \qquad \text{Equation 3.2}$$

The total basic reproduction number (Q_0) including projected development of eggs shed by adult worms ($Q_{0(d)}$) and projected development of larvae surviving from the previous autumn ($Q_{0(s)}$), in the absence of immunity, is described by

Model 1: $$Qo = \frac{Pd(T)\lambda\beta Hq + Ps(T)Pw(T)\lambda\beta Hq}{(\mu 1(T) + \beta H)\mu 2}$$ Equation 3.3

with the contribution of development of eggs to total Qo, at any given day, amounting to

Model 2: $$Qo(d) = \frac{Pd(T)\lambda\beta Hq}{(\mu 1(T) + \beta H)\mu 2}$$ 'Development model' Equation 3.4

and the contribution of larval survival

Model 3: $$Qo(s) = \frac{Ps(T)Pw(T)\lambda\beta Hq}{(\mu 1(T) + \beta H)\mu 2}$$ 'Survival model' Equation 3.5.

3.3 Parameterisation

Parameterisation proved possible for the best studied species, *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*, only. For the other economically important species, *Nematodirus battus*, even basic data (such as the threshold for development of eggs) was found to be lacking. Also, for this species, the presence of infective larvae at pasture depends not only on larval development and death rates but also on the hatching of eggs, a

Parameter	Definition	Assumptions/remarks
$P_{d(T)}$	Probability that an egg develops into an infective larva at temperature T	Development dependent on temperature only
$P_{s(T)}$	Probability that eggs deposited in the last two weeks of the previous September have developed into infective larvae	Based on the mean development temperature measured in those weeks
$P_{w(T)}$	Probability that an infective larva present the previous October 1st is still alive	$P_{survival}= 1-\Sigma(\mu_{l(t)}-\mu_{l(October1st)})$
λ	Mean rate (eggs/day) at which adult parasites produce eggs	Non-immune host
β	Rate at which larvae are eaten by a single lamb (as the proportion of total available herbage on one hectare/day)	Larvae evenly distributed over pasture and over herbage
H	Host density (lambs/hectare)	-
q	Probability that an ingested L3 develops into an adult parasite	Non-immune host
$\mu_{l(T)}$	Temperature-dependent instantaneous daily mortality rate of infective larvae (proportion/day)	No other noxes contributing to larval death
μ_2	Mortality rate of adult parasites (proportion/day)	Non-immune host

Table 3.1 Definitions and assumptions of model parameters; (T) shows the modelled parameters are in some way a function of current or historic temperature; Parameters without (T) are constants.

process which has its own temperature requirements and which occurs only once or twice a year (Gibson and Everett, 1981). Such a species, therefore, does not appear to be suited to the throughout-year modelling of daily predicted success. For these reasons, only *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* were modelled.

Probability that an infective larva present October 1st of the previous year is still alive - $P_{w(T)}$

The modelling of the probability that a cohort of infective larva present the previous October 1st is still alive on day *t* of the next year ($P_{w(T)}$), was done in a novel way (table 3.1) . Estimation of the number (or proportion) of larvae alive at pasture at time *t* is normally achieved by multiplying constant instantaneous daily survival rates (e.g. Grenfell *et al.* 1986). This method has been shown to adequately represent mean larval densities for populations of larvae consisting of several cohorts with an unknown history (Smith and Grenfell, 1994) but is likely to misrepresent the survival of one cohort of freshly developed larvae. Also, this study aims to explicitly explore the influence of temperature on the survival of larvae.

Therefore, it was assumed that larvae will die when their energy reserves are depleted and that energy reserves are more rapidly depleted with increasing temperatures while frost, above a certain threshold, may irreversibly alter reserves. The mean level of energy reserves of a cohort was assumed to represent the probability of the larvae to be alive. Larval death rates were made a function of temperature (see below) and the individual contributions of days at temperature (T) to the depletion of energy reserves summed and deducted from the starting value (1).

Host stocking density- H

Compared to the countries for which the stocking density was surveyed by Kao *et al.* (2000) typical UK stocking densities are much higher and more in line with a Danish example of 17 ewes, plus their lambs, per hectare given by Thamsborg *et al.* (1996b). Stocking density was set at 50 weaned lambs per hectare.

Larval ingestion rate – β

After Kao *et al.* (2000) it was assumed that larvae are evenly spread over pasture. The daily larval ingestion rate could now be calculated as the daily grazing rate per individual animal divided by the available herbage. The dry-matter herbage intake for lambs just after weaning was set at 0.8 kg per day (Paton *et al.* 1984) and the average UK kg dry herbage at pasture at 2200 (Waller *et al.* 1981), making β 3.6 x 10^{-4}. In reality the value of β will be determined by the month, or the season, of the year. However, as the model determines the daily projected success of the worm population at pasture infected weaned lambs have just been turned out on, it is quite realistic to work with a set value. Farmers are likely to, regardless of the time of the year, wait until the grass is a certain length before lambs are allowed to graze it.

Probability of eggs developing into infective larvae at a given temperature - $p_{d(T)}$ and $p_{s(T)}$

Both Coyne and Smith (1992) and Rossanigo and Gruner (1995) incubated a large number of dung sample replicas at a variety of temperatures and estimated the developmental success making use of a simple Baermann technique. Rossanigo and Gruner (1995) worked on, amongst other species, *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *Haemonchus contortus* but the data are not presented for the latter species. Proportions of *Haemonchus* eggs developing into L3 were therefore estimated from the Coyne and Smith (1992) study.

T. circumcincta and T. colubriformis

Rossanigo and Gruner (1995) worked with narrow temperature intervals and it is very clear from their data that the developmental success of both species slowly increases towards the optimum temperature but drops steeply when the temperature increases further. Therefore it was decided to fit two separate linear regression lines, one for the temperature range up to the optimum and one for temperatures past the optimum. The mean and standard deviations, each the result of ten replicas, were reconstructed from the paper. For each distribution ten data points were randomly simulated and the regression line was fitted through these data points. The data points and regression lines are shown in figure 3.1 and the regression equations in table 3.2. The minimum threshold for development was set at 4°C for *T. circumcincta* (Crofton, 1965; Young *et al.* 1980) and 5°C for *T. colubriformis* (Beveridge *et al.* 1989). The slopes of the regression lines do not overlap and this indicates that *T. colubriformis* will, relatively speaking, benefit more from temperature increases up to the optimum development temperature than *T. circumcincta* but also suffer relatively higher losses at temperatures over the optimum.

	T_{min} to T_{opt} (95% CI slope)	R^2 (Tmin to Topt)	T_{opt} to T_{max} (95% CI slope)	R^2 (Topt to Tmax)
T. circumcincta	0.084 + 0.014*T (0.0032)	0.56	1.24 -0.0352*T (0.0052)	0.79
T. colubriformis	-0.051 + 0.013*T (0.0019)	0.69	2.25-0.067*T (0.0064)	0.93
H. contortus	-0.944 + 0.114*T 0.002*T^2	0.32	As T_{min} to T_{opt}	As T_{min} to T_{opt}

Table 3.2 Equations describing regressions of the proportion of eggs successfully developing into infective larvae a temperature T. T_{min}=minimum development threshold, T_{opt}=temperature at which development is optimal, T_{max}=upper development threshold.

H. contortus

Coyne and Smith (1992) examined a very large number of samples at a wide variety of incubation periods. They concluded that, rather than one optimal temperature, there is an optimum range, 20-30°C, for *H. contortus* development. The same range emerges from field experiments presented by Levine *et al.* (1974) and even from laboratory experiments published in 1916 (Veglia, 1916, as cited by Anderson, 2000). For this reason it was decided that a polynomial line would provide a more appropriate fit for the *Haemonchus* data. For each temperature setting, data points were extracted at the time of maximum larval recovery. Data points and regression line are given in figure 3.1 and the regression equation in table 3.2. A point estimate mean proportion of larval recovery of 0.485 at 23°C given by Rossanigo and Gruner (1995) closely fits that predicted by the model (0.504). The minimum temperature for development was set at 11°C (Gibson and Everett, 1976a; Besier and Dunsmore, 1993). 40°C is the upper limit for *Haemonchus* development (Misra and Ruprah, 1973; Jehan and Gupta, 1974) but the model does not provide any information for development above 35°C. The maximum corrected mean daily soil surface temperature over the past 30 years at any of the three weather stations used for the modeling was 26.5°C, well within the limits of the model. The highest *maximum* daily soil surface temperature was 37°C, showing that *Haemonchus* development, in the UK, is currently unlikely to be limited by temperatures towards the upper threshold at any time during the summer.

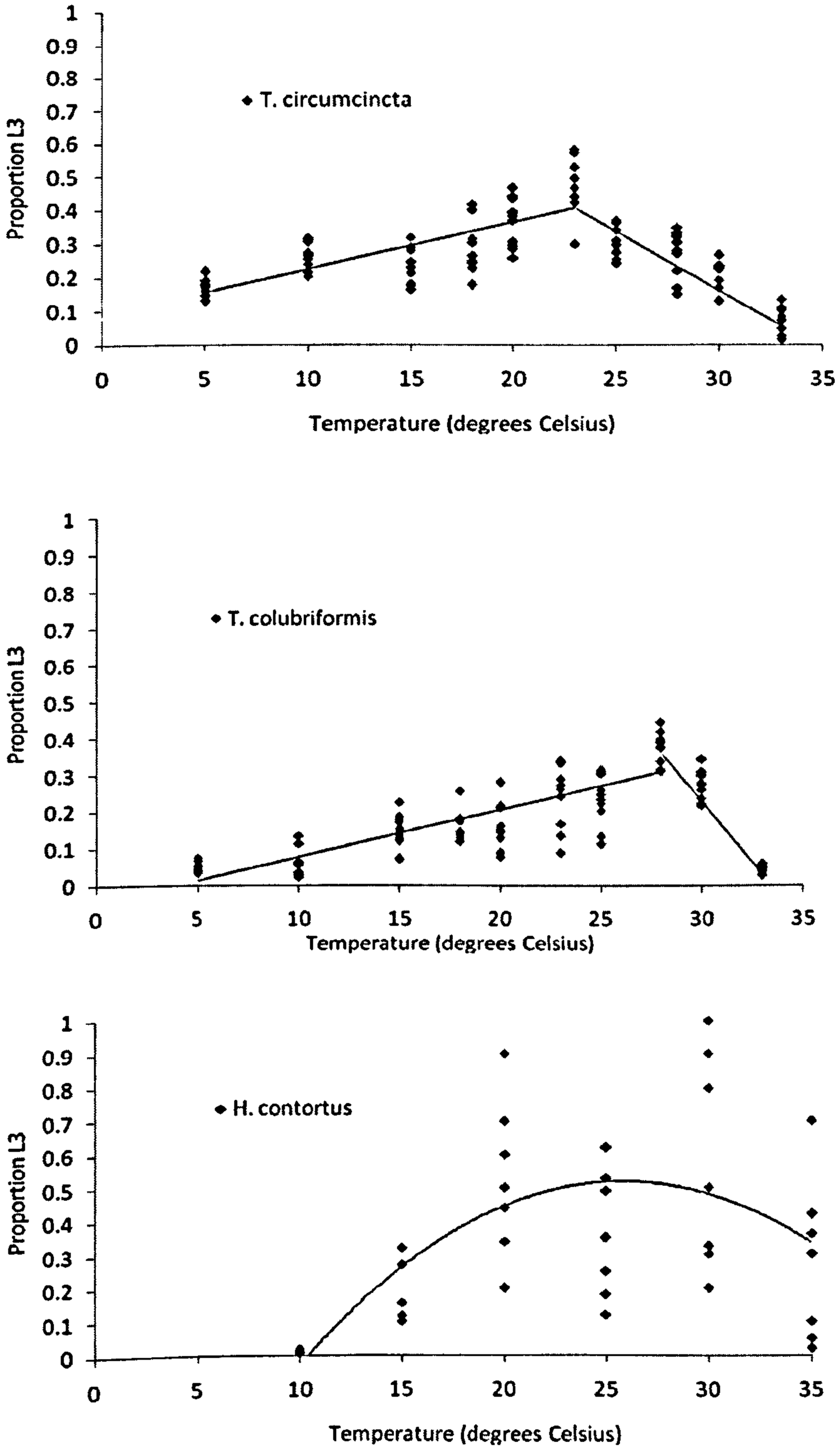


Fig 3.1 The proportion of eggs recovered as L3 at different temperatures for *T. circumcincta, T. colubriformis* and *H. contortus*. Data points simulated from Rossanigo and Gruner (1995) and extracted from Coyne and Smith (1992); the lines represent regressions.

Larval death rate as a function of temperature - $\mu_{l(T)}$

Instantaneous daily mortality rates were estimated from relevant published laboratory survival studies. Numbers of larvae surviving at given times of exposure to constant temperatures were extracted and the rates estimated as the slopes of the log-linear survival regression lines. Point estimates of larval survival of all three species published by Boag and Thomas (1985) were added as data points for model estimation, as 1/median survival time. For *T. colubriformis*, data from work on the sister species *Trichostrongylus vitrinus* by Rose and Small (1984) was included. After this process, for each of the three species under study, 12-15 data points were available.

Determined temperature-dependent mortality rates were, especially at lower temperatures, surprisingly low. Also, the studies had worked with various timescales. Therefore, studies carried out over one year would find measurable decay of larvae where studies examining larvae for a few months only sometimes did not. Therefore the need was felt to compare the larval death rates established from the literature with those established at a timescale at which, in the UK, larvae would experience those temperatures at pasture. Lastly, the influence of temperature drops below zero (night frosts), on larval survival had not been established while very few data points on continuous temperatures just below zero were available. Therefore, L3 of all three species were obtained from the Moredun Research Institute and three replicas of batches of larvae kept in filtered water and exposed to -4, 4 and 11°C continuously, and 4 and 11°C continuously but with one weekly 'night frost'. The latter treatments were, once a week, manually removed from their incubators, put at -2°C overnight, and put back in the morning of the following day. Biweekly approximately 100 larvae of each replica were examined and non-motile, stretched-

out, larvae counted as dead. After examination larvae were discarded. All treatments were followed for 120 days. For estimation of larval death rates numbers of live larvae per 100 larvae examined were log transformed and, in order to establish whether regression was possible, tested for significance in decreases over time using Pearson rank correlation.

As can be seen in table 3.4 below only the *Haemonchus* -4°C treatment was significantly negatively correlated with time. The slope of the regression line was -0.032 (95% CI +/- 0.014). The 4°C + frost treatments of *T. colubriformis* and *H. contortus* were significantly positively correlated with time but the slope of the regression line was less than 0.0001.

It appears that UK temperature-related larval decay is, over a time frame approximating the winter season, likely to be negligible for *T. circumcincta* and *T. colubriformis*. For *Haemonchus*, a weekly night frost of -2°C does not have any measurable detrimental effect on L3. This finding is in line with the only published study cycling larvae over a similar range: Troell *et al.* (2005), measuring larvae over a period of 24 weeks, found very low mortality rates in *Haemonchus* larvae undergoing daily 12-hourly cycles of temperatures between -1 and 15°C. However, as temperatures fall further μ rises rapidly for this species. At -10°C, Todd *et al.* (1976) found daily rates approximating -0.99.

For *T. circumcincta* and *T. colubriformis*, the data points of the 4 and 11°C treatments were added to the pool of rate estimates and these are given in figure 3.2. As can be seen the rates increase rapidly above 30°C and at 45°C virtually all larvae die within one day (Andersen and Levine, 1968; Misra, 1978). However, as mentioned above, UK mean daily soil surface temperatures do not reach this level and therefore only rates established at 30°C or below

	T. circumcincta	*T. colubriformis*	*H. contortus*	r_p	p
4°C	*NS*	*NS*	*NS*	≤ +0.303 ≥ -0.026	≥ 0.150
11°C	*NS*	*NS*	*NS*	≤ +0.339 ≥ -0.299	≥ 0.106
4°C + frost	*NS*	*SP*	*SP*	≤ +0.419 ≥ +0.371	≥ 0.043
11°C + frost	*NS*	*NS*	*NS*	≤ +0.130 ≥ -0.224	≥ 0.292
-4°C	*NS*	*NS*	**SN**	≤ 0.442 ≥ -0.824	≥ 0.001

Table 3.3 Pearson rank correlation of larval survival with time. NS=not significantly correlated, SP=significantly positively correlated, SN= significantly negatively correlated. r_p= Pearson rank correlation coefficient.

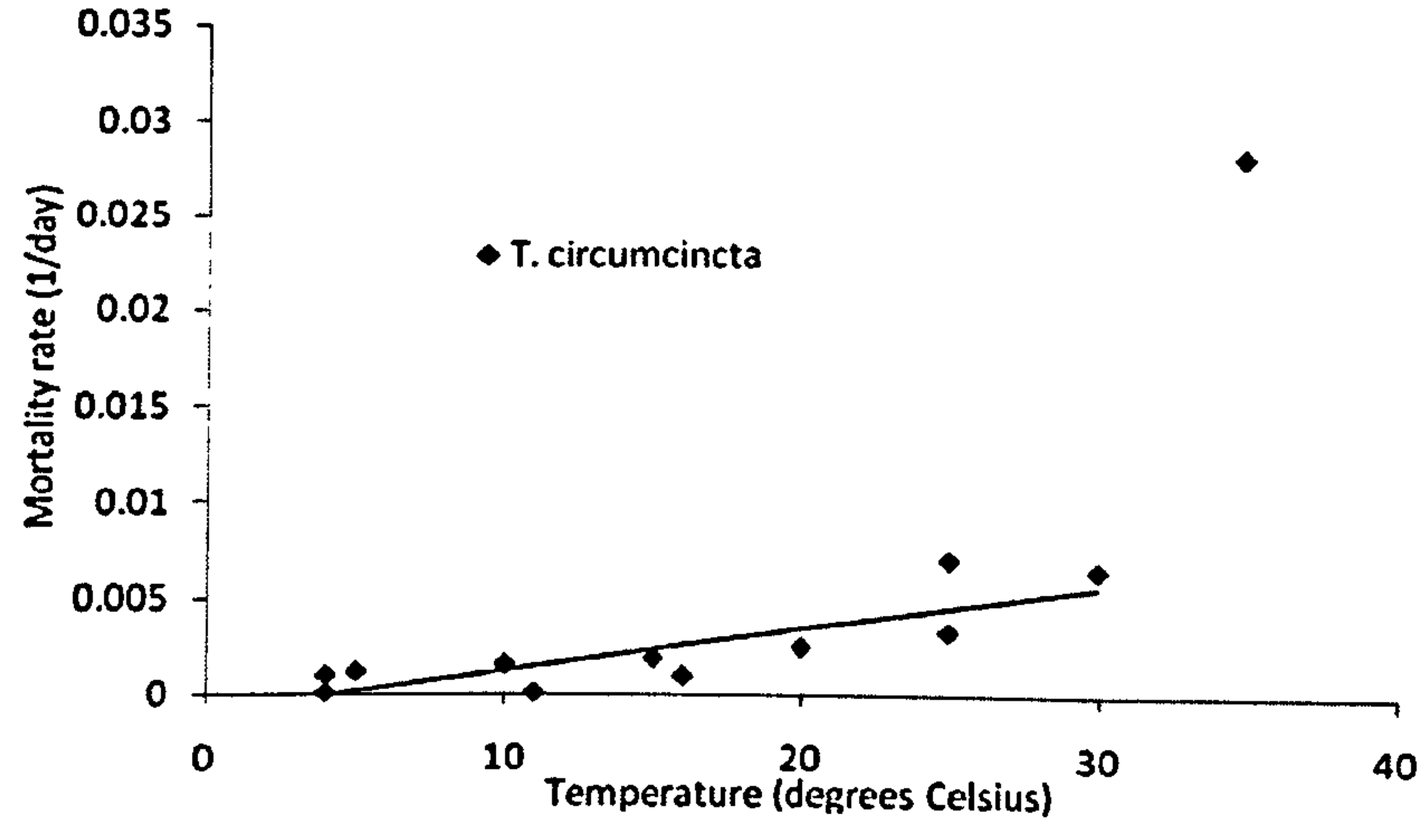
were included for model estimation. For all three species the rates increased significantly with increasing temperature ($r_s \geq 0.795$, $p \leq 0.001$). Linear and exponential models were fitted to the data and, as both models fitted the data very well for all three species ($X^2_{(\geq 12)} \leq 10.50$; $p \geq 0.572$) there was no advantage in applying the quadratic model. Very few larval death rates for temperatures below zero were available in the literature (fig. 3.2 D&E). As the findings presented above had shown that *T. circumcincta* and *T. colubriformis* were both able to withstand -4°C for four months, and constructed rates at -10°C were very similar, it was assumed that, at -4 to -10°C, survival of both species was identical. For the *Haemonchus* data, quadratic and polynomial models severely underestimated the rates at the main UK temperature range of interest (0 to -6°C). It was decided to simply fit a linear model to the information available and to

revisit the matter if model output indicated that over winter survival was critical for the success of the parasites in the following year. The linear regression equations are given in table 3.4.

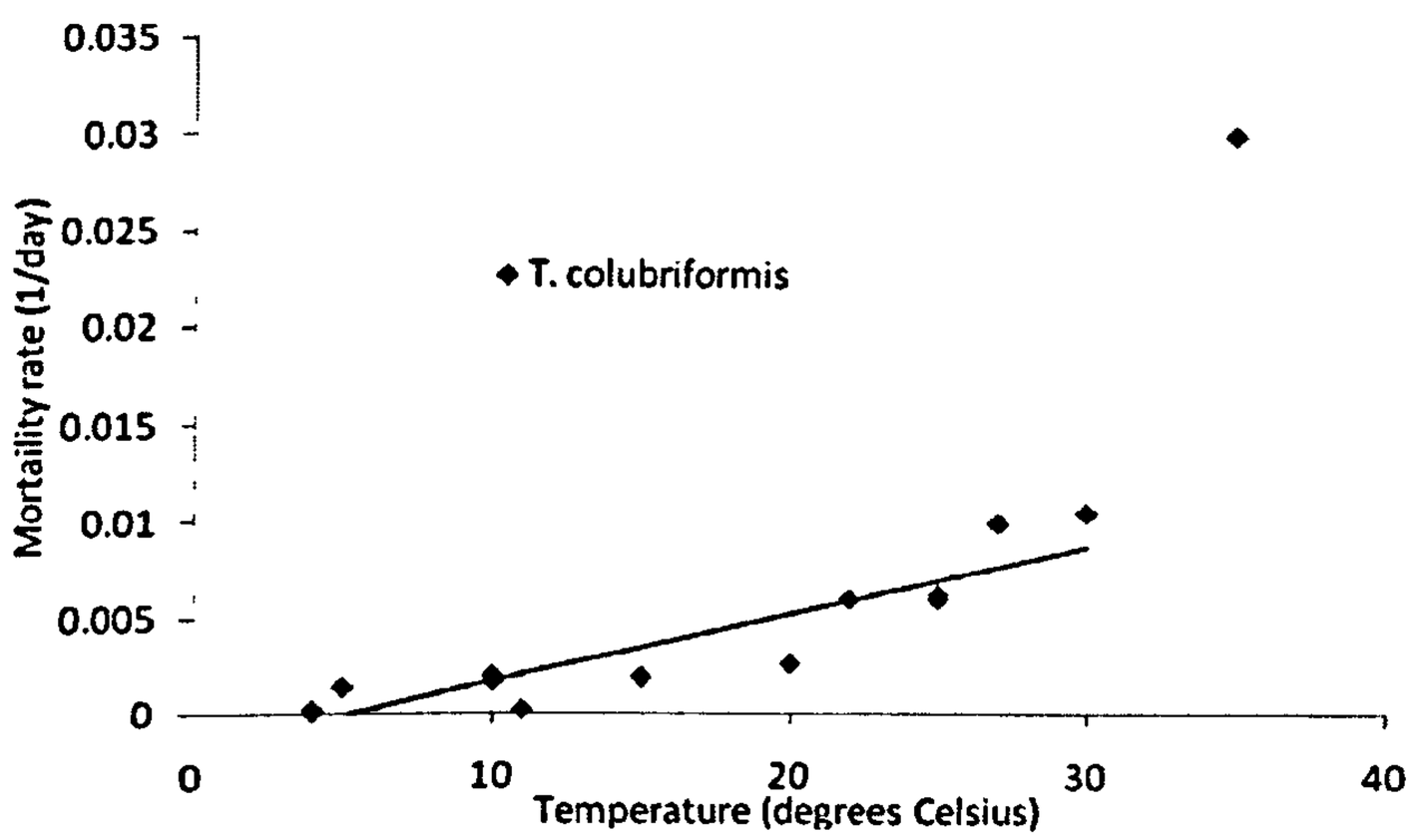
Species	Range	Regression equation	95% CI slope (+/-)
T. circumcincta	4 to 30°C	-0.00088 + 0.000217* T	0.00010
T. colubriformis	4 to 30°C	-0.00181 + 0.000351* T	0.00009
T.circumcincta & T. colubriformis	-4 to -10°C	-0.0456 + 0.0114 * T	0.010
H. contortus	4 to 30°C	-0.00127 + 0.000462 *T	0.00020
H. contortus	-1 to -7°C	-0.0194 – 0.0161 * T	0.012

Table 3.4 Linear regressions of instantaneous daily larval death rates on temperature.

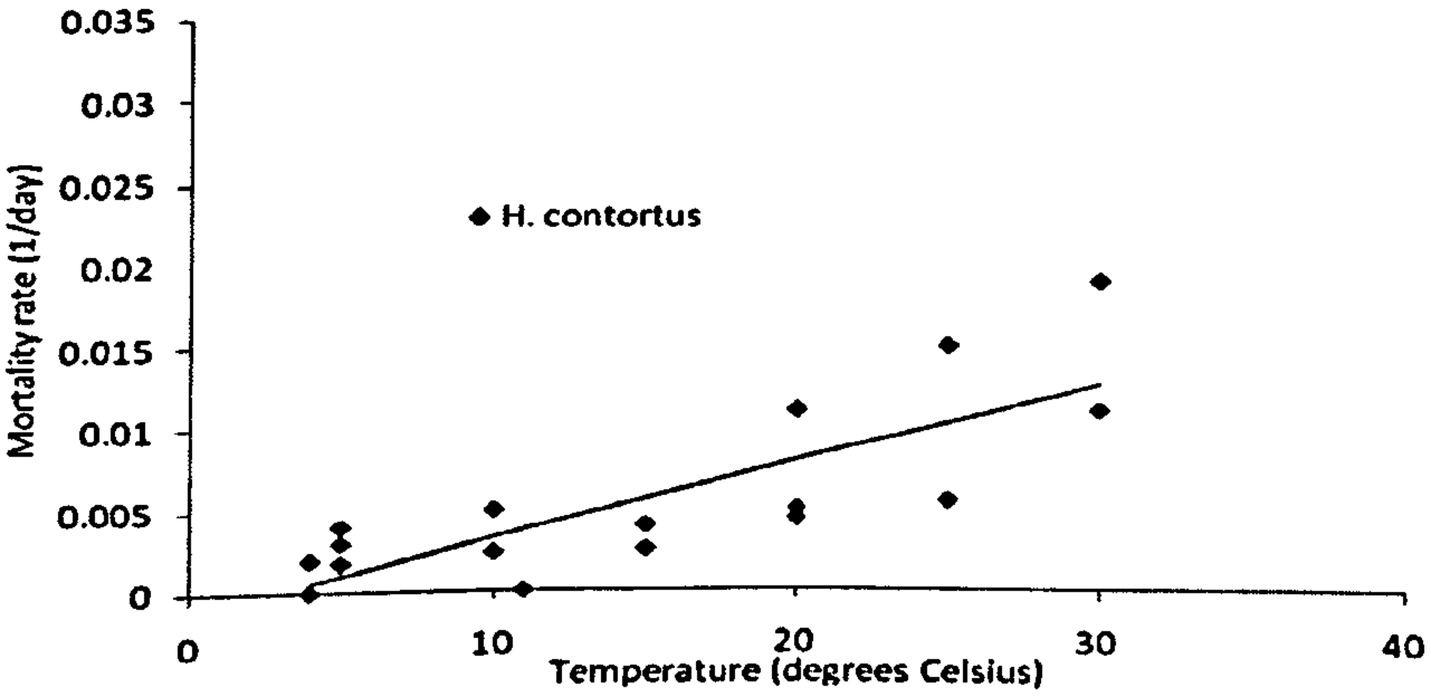
For all three species the minimum larval death rate at approximately 4°C, and below this temperature, was zero. This fits the laboratory data presented above. From Pandey *et al.* (1993) a rate estimate of 0.002 was extracted for *T. circumcincta* kept at -10°C continuously. As the coldest mean daily soil surface temperature during the 30 years under study was -8.1 (and the lowest minimum daily temperature -12.3) it appears that a model assuming all larvae to survive the winter at temperatures below zero is justified, especially as larvae in soil are more likely to survive frost than larvae kept in water (Anderson and Levine, 1968).



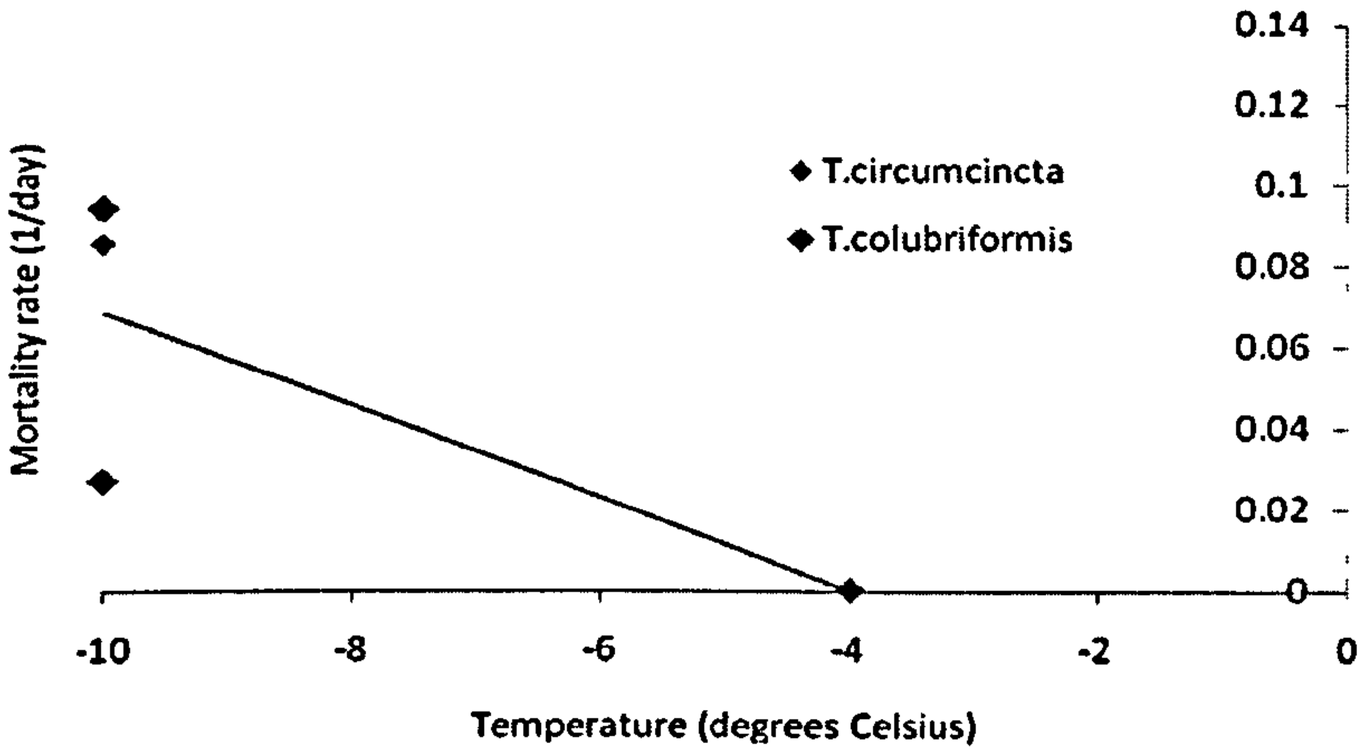
A. *T. circumcincta*. Data points from Boag and Thomas (1985), Pandey *et al.* (1993) and the present study.



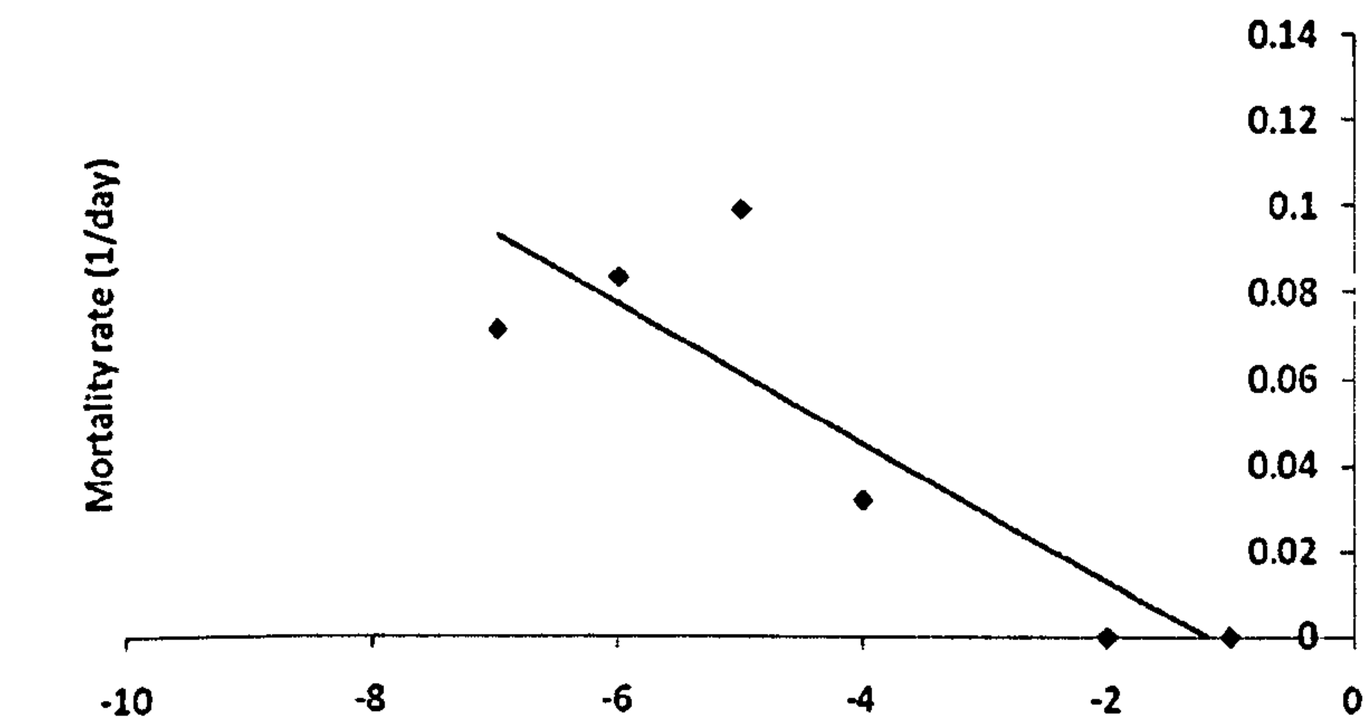
B. *T. colubriformis*. Data points from Anderson and Levine (1968), Rose and Small (1984), Boag and Thomas (1985) and the present study.



C. *H. contortus*, 4 to 30°C. Data points from Todd *et al.* (1976), Misra (1978), Boag and Thomas (1985), Troell *et al.* (2005) and the present study. Above 30°C, the rate increases sharply to 0.145 at 40°C (not pictured).



D. *T. circumcincta* and *T. colubriformis*, -4 to -10°C. Data points from Anderson *et al.* (1966), Andersen and Levine (1968), Pandey et al. (1993) and the present study.



E. *H. contortus*, -1 to -7°C. Data points from Rose (1963), Todd *et al.* (1976), Philip (1983), Troell *et al.* (2005) and the present study.

Fig. 3.2 A-E Larval death rates at various continuous temperatures. The lines represent regressions.

Establishment rate of infective larvae in the host – q

From the relevant studies detailed in the survey by Kao *et al.* (2000), carried out at various dose rates, the mean proportions of infective larvae retrieved as adult worms were calculated as 0.41

(*T. circumcincta*), 0.43 (*T. colubriformis*) and 0.53 (*H. contortus*). Similar values (approx. 0.50) were also obtained for the species *N. battus* (Lumley and Lee, 1981; Winter *et al.* 1996). The establishment rate was therefore assumed to be similar and set at 0.50 for all three species.

Death rate of adult parasites - μ_2

Leathwick *et al.* (1997) published the only study comparing death rates of adult worms of two species in one set-up. In this study the daily proportion loss of *T. circumcincta* and *T. colubriformis* adult worms, in the absence of immunity, was established as approximately 0.11 and 0.05, respectively. Coyne *et al.* (1991) found a value of 0.12 for *H. contortus*. Given the error margins of the two studies, and the scantiness of data, μ_2 was set to 0.10 for all three species

Mean rate of egg production per adult worm – λ

A detailed overview of published daily egg production rates is given in Kao *et al.* (2000). Mean rates were approximated at 170, 300 and 3300 per adult worm for *T. circumcincta*, *T. colubriformis* and *H.contortus*, respectively.

An overview of the values of the parameters and thresholds for the three species used in the model is given in table 3.5.

Parameter	*T. circumcincta*	*T. colubriformis*	*H. contortus*
T_{min}	4 °C	5 °C	11 °C
$P_{d(T)}$	linear functions of temperature (table 3.2)	linear functions of temperature (table 3.2)	polynomial function of temperature (table 3.2)
$P_{s(T)}$	the value of $P_{d(T)}$ for the mean temperature 17^{th} –30^{th} September of the previous year	the value of $P_{d(T)}$ for the mean temperature 17^{th} –30^{th} September of the previous year	the value of $P_{d(T)}$ for the mean temperature 17^{th} –30^{th} September of the previous year
$P_{w(T)}$	1 – (summed daily instantaneous larval death rates October 1^{st} – day (t))	1 – (summed daily instantaneous larval death rates October 1^{st} – day (t))	1 – (summed daily instantaneous larval death rates October 1^{st} – day (t))
λ	170 / worm / day	300 / worm / day	3300 / worm /day
B	3.6×10^{-4} / day	3.6×10^{-4} / day	3.6×10^{-4} / day
H	50 / hectare	50 / hectare	50 / hectare
q	0.50	0.50	0.50
$\mu_{1(T)}$	linear functions of temperature (table 3.4)	linear functions of temperature (table 3.4)	linear functions of temperature (table 3.4)
μ_2	0.10 / day	0.10 / day	0.10 / day

Table 3.5 Overview of Q_0-parameter values and thresholds used for the species *T. circumcincta*, *T. colubriformis* and *H. contortus*. T_{min} is the minimum development threshold; the other symbols are explained in table 3.1.

3. 4 Explorations of the model and analysis

The model was run in one-day time steps. To this purpose, 30-year (1977-2006) daily mean air temperature data were purchased from the MET Office. Three MET office weather stations for which a complete data set was available were Yeovilton, Waddington and Paisley. It was assumed that these stations adequately represented the sheep-dense areas of the Southwest, the Midlands and Scotland, respectively. The mean daily temperatures were transformed into soil surface temperatures as described in the Appendix. For stochastic simulations, it was assumed that daily mean temperatures follow the normal distribution and the 30-year mean daily temperature, and its standard deviation, was computed.

3.4.1 Validation

The Q_0 model predicts parasite offspring in the absence of host immunity and therefore it cannot truly be validated against the abundance data presented in chapter 2. However, if data on disease of the host closely resembles that of predicted success this indicates that increases in parasitic abundance lead to a higher disease rate in certain hosts, regardless of their immune status, and this would be valuable information.

For each weather station, the model was run on 29 years of data (as the temperatures of the previous September have to be included for each year) and the total of predicted offspring summed. For each run (in this and all following parts) the total predicted success (Model 1), the predicted success from larval development during the year (Model 2), and the predicted success resulting from the consumption of larvae that had survived from the previous October (Model 3)

were recorded separately. The mean of predicted Q_0 values for *T. circumcincta* and *T. colubriformis* were chosen to represent the category 'NOS'. Summed annual Q_0 values were divided by 365 to give the average Q_0/year, a measure of the success of a parasite in that year . The predicted annual success of the categories NOS and Haemonchosis was plotted against the annual diagnostic rate (expressed as a percentage of diagnosable submissions, chapter 2) of each of the three regions, and tested for Spearman-rank correlation.

The total monthly predicted success was also recorded. It was summed for the three regions and the monthly contributions to the total of predicted success for each year computed for the three regions. These were compared to the distribution of diagnosed outbreaks over the year in Great Britain and the goodness of fit tested by means of a Chi-square test.

The annual predicted success of the NOS and Haemonchosis category parasites were tested for trends, and significance in differences between regions, in a Jonckheere test and post-hoc tests as described in chapter 2. The same was done, on summed values for the three regions for each year, to compare differences in the predicted success between the three species in the UK.

3.4.2 Sensitivity analysis

With respect to the parameter $P_{d(T)}$, the value of Q_0 follows the pattern shown in fig. 3.1. For the other modeled parameters, Q_0 follows alterations to these in a linear fashion. Changes in μ_1 not only impact linearly on Q_0 but also through P_w, and therefore this parameter was singled out for sensitivity analysis of the model. The influence of increasing $\mu_{1(T)}$ up to ten fold ('Mu 2' –'Mu 10') on the output of models 2 and 3 was explored running these models on 30-year mean daily data for the regions Southwest and Scotland. In order to be able to describe confidence limits, the

influence of μ_1 on the total annual Q_0 , and the contribution of survival-related Q_0 to the total annual Q_0, was explored stochastically on 30-year daily mean data and their standard deviations . Predicted offspring the result of larval over winter survival 1978-2006 was compared between Scotland and the Southwest, for Mu levels 1 and 3, in Mann-Whitney U tests. The Bonferroni-adjusted significance level was set at 0.008.

3.4.3 Climate change

The influence of (further) increases in temperature on the contribution of 'survival Q_0' to total annual Q_0, and on total annual Q_0 itself, was explored stochastically by increasing the mean in 0.5°C steps. This is unlikely to represent a realistic climate change scenario but was done to explore general likely trends. The mean total Q_0 over 1977-2006 was taken as the nil-point and Q_0 after temperature increases expressed as a proportion of this. Lastly, the influence of temperature increases on monthly predicted Q_0 was modelled at several levels of μ_1 (laboratory μ_1 (Mu1), Mu3 and Mu5).

3.5 Results

3.5.1 Validation

In figures 3.3 and 3.4 the predicted success, presented as the annual number of offspring, is plotted against the diagnostic rate. Data were transformed to normalise the distribution. NOS diagnostic rates, and Haemonchosis Q_0, were right-skewed and log-transformed. Haemonchosis

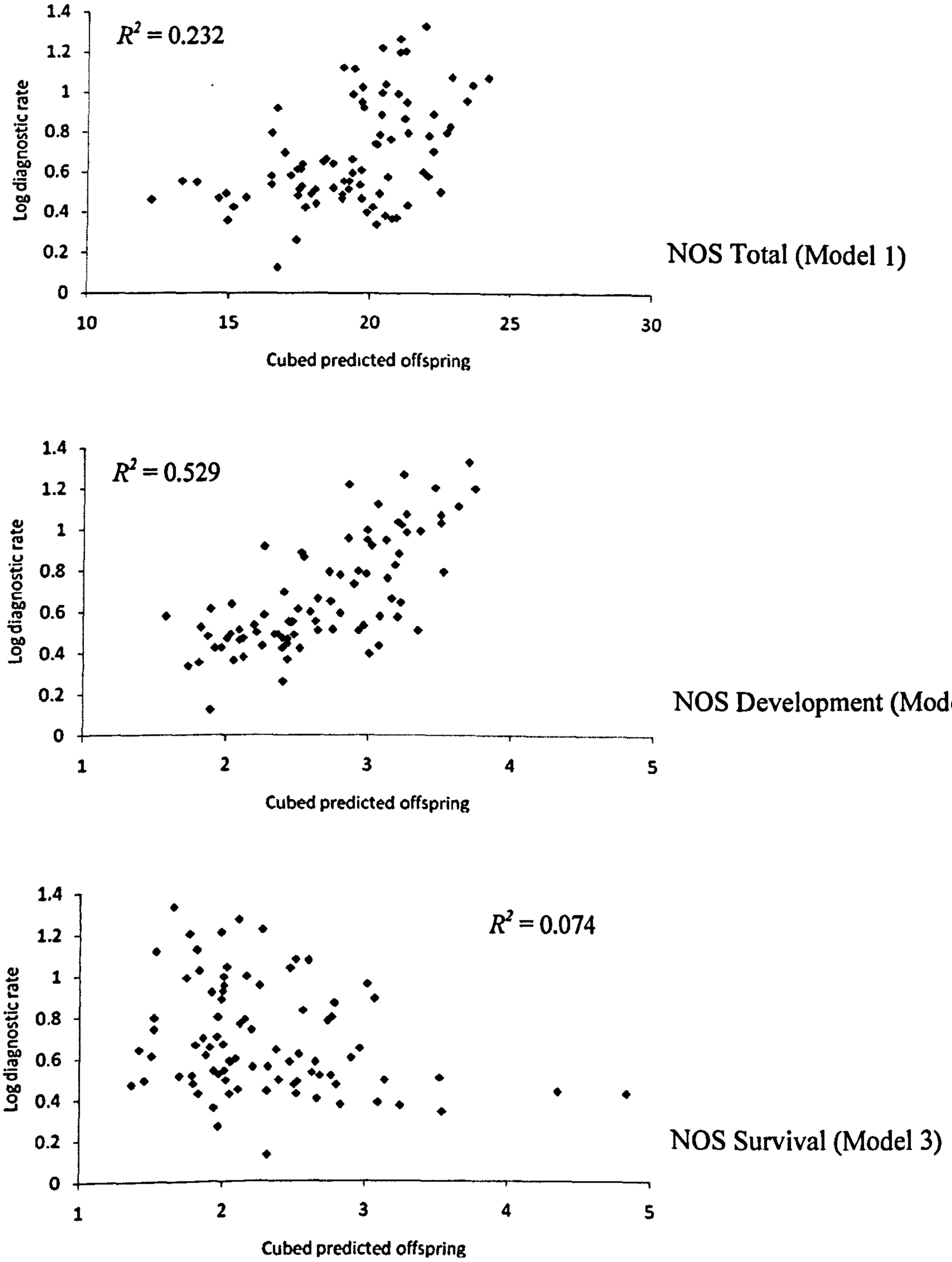


Fig. 3.3 Cubed mean predicted annual success (offspring/worm / 10^6) of worms in the category NOS plotted against Log diagnostic rate, over 1978-2006 for the regions Southwest, Midlands and Scotland. NOS total represents output of Model 1, NOS development output of Model 2 and NOS survival that of Model 3. The scale of the horizontal axes is identical for NOS Development and NOS Survival; the NOS Total graph is on a different scale.

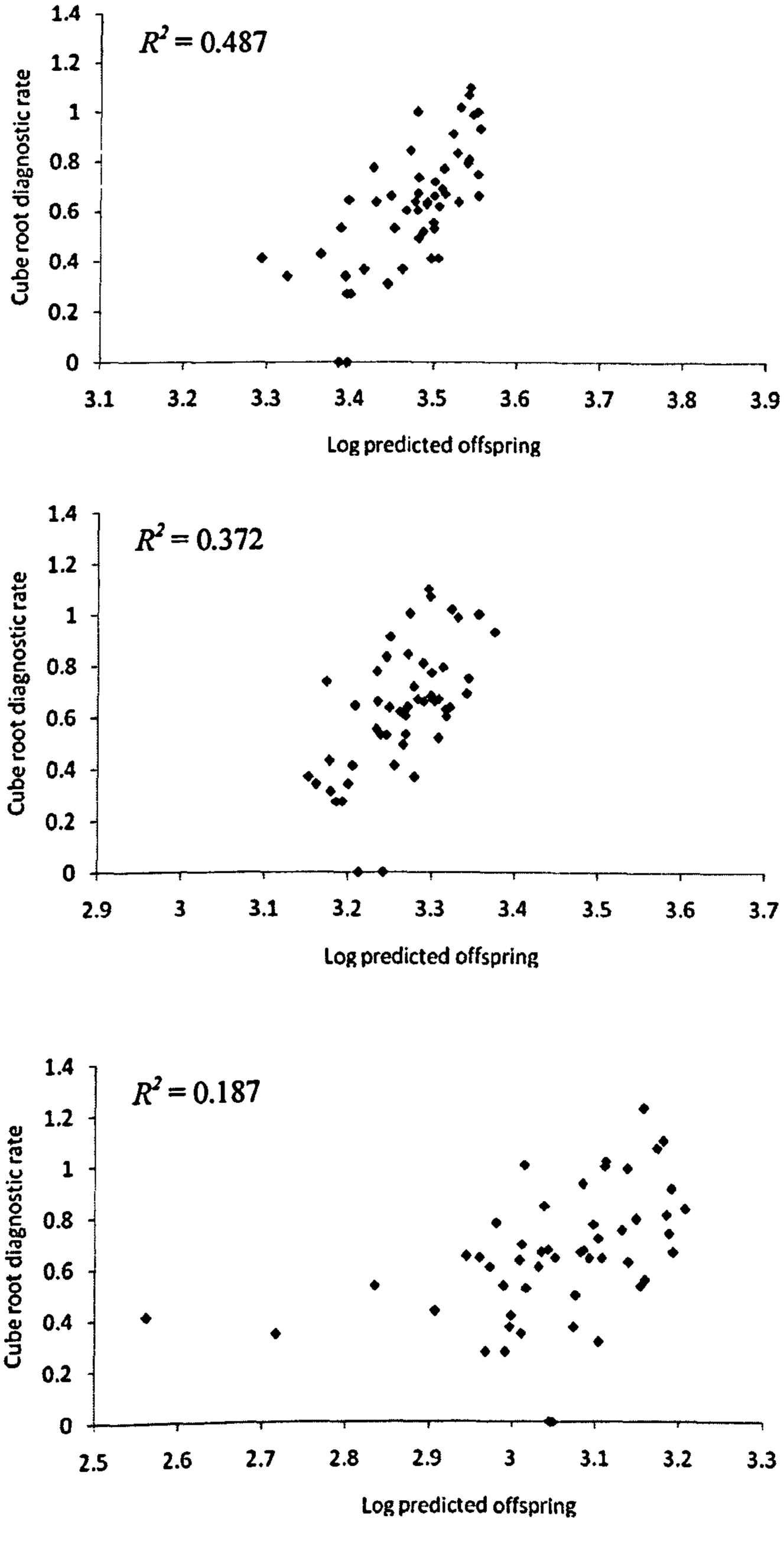


Fig. 3.4 Log mean predicted annual success (offspring/worm) of worms in the category Haemonchosis plotted against the cube root of the annual diagnostic rate, over 1989-2006, for the regions Southwest, Midlands and Scotland. Haemonchus Total represents the output of Model 1, Haemonchus Development that of Model 2,and Haemonchus Survival that of Model 3.The scale of the horizontal axes has been set so that their range is identical on the three graphs.

diagnostic rates were also right-skewed but did include zero values, while values were too small to log(value +1) transform, and were therefore cube root transformed. NOS Q_0 was left-skewed and cube transformed. For both categories, NOS and Haemonchosis, disease incidence is associated with model-predicted parasite success. For the category NOS, years with high disease incidence have been experienced in years with high developmental success of eggs while over-winter survival-related success has tended to be lower in those years. For Haemonchosis, apart from developmental success (Model 2), larval over winter survival (Model 3) is also positively correlated with disease rates. The correlations were significant for all but the NOS survival model (Model 3; see table 3.6)

	NOS Model 1	NOS Model 2	NOS Model 3	**Haemonchosis Model 1**	Haemonchosis Model 2	Haemonchosis Model 3
r_s	0.475	0.715	-0.259	0.725	0.612	0.543
p	<0.001	< 0.001	0.017	< 0.001	< 0.001	< 0.001

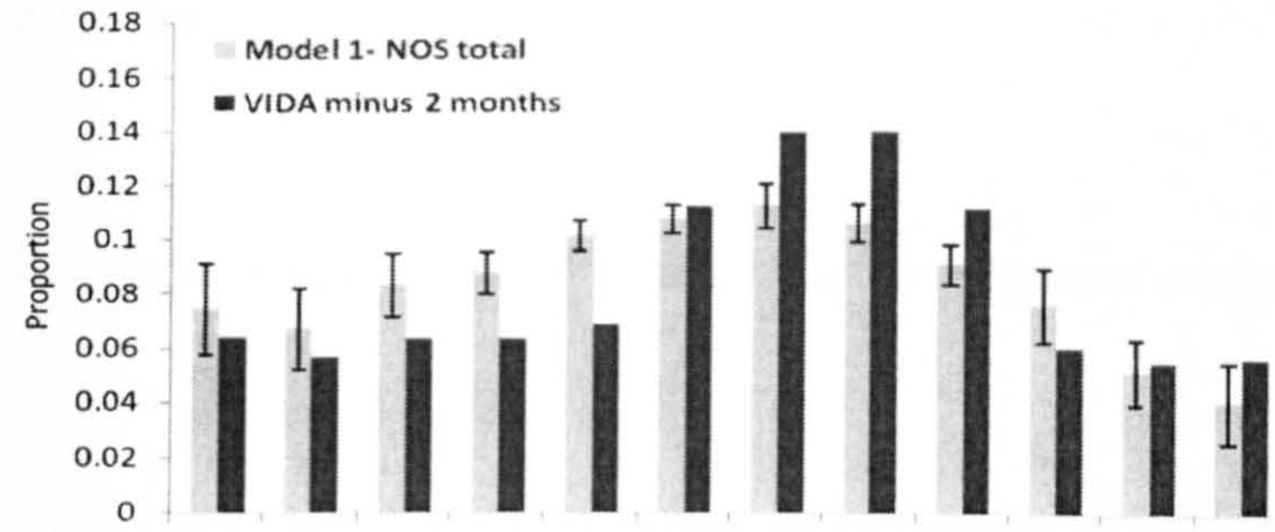
Table 3.6 Spearman correlation coefficients and p values for the strength of correlation between diagnostic rate and parasitic success predicted by the models. The Bonferroni-adjusted significance level is 0.008.

Comparing the relative importance assigned to different months by the model with the actual importance of months in terms of disease, model predictions resembled actual events closely. However, for the category NOS disease outbreaks showed a 2-month time lag on increases of predicted parasitic success and for the category Haemonchosis a 1-month time lag showed the closest resemblance to actual events (fig 3.5) Deducting these time lags, for both NOS model 1 and NOS model 2, there were no significant differences between model-predicted and VIDA mean values (Model 1: $X^2_{(11)} = 6.480$, p = 0.840; Model 2: $X^2_{(11)} = 5.633$, p = 0.897). For

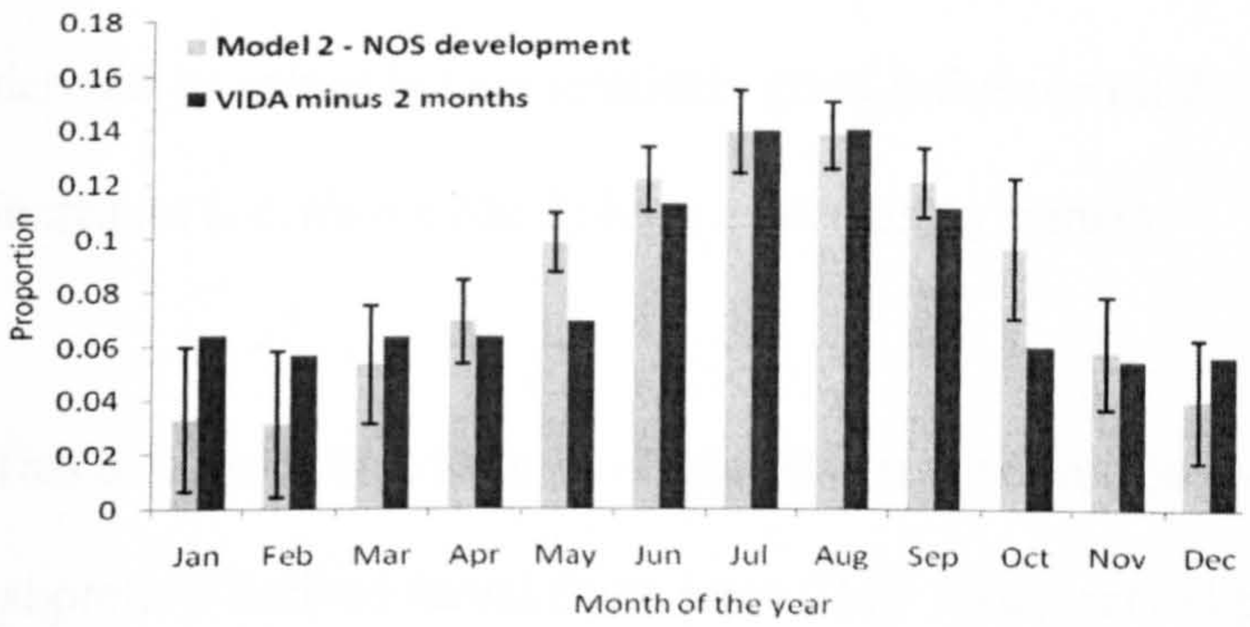
Haemonchosis, neither model predicted outbreaks significantly well, both underestimating the 'peakyness' of the outbreaks. The chi-squared value for the fit of model 2 was half that of model 1 (Model 1: $X^2_{(11)} = 51.55$, $p < 0.0001$; Model 2: $X^2_{(11)} = 25.41$, $p = 0.008$).

In agreement with the trends described in chapter 2, for both categories, NOS and Haemonchosis, significant decreasing trends in predicted regional success were found in the order Southwest – Midlands – Scotland ($J = 880$, $z = -2.973$, $p = 0.003$ and $J = 263$, $z = -3.54$, $p=0.001$, respectively). For both categories differences between the Southwest and the Midlands were not significant ($U \geq 159$, $z \leq -0.661$, $p \geq 0.524$) but differences between Scotland and the other two regions were ($U \leq 263$, $z \geq -2.449$, $p \leq 0.014$).

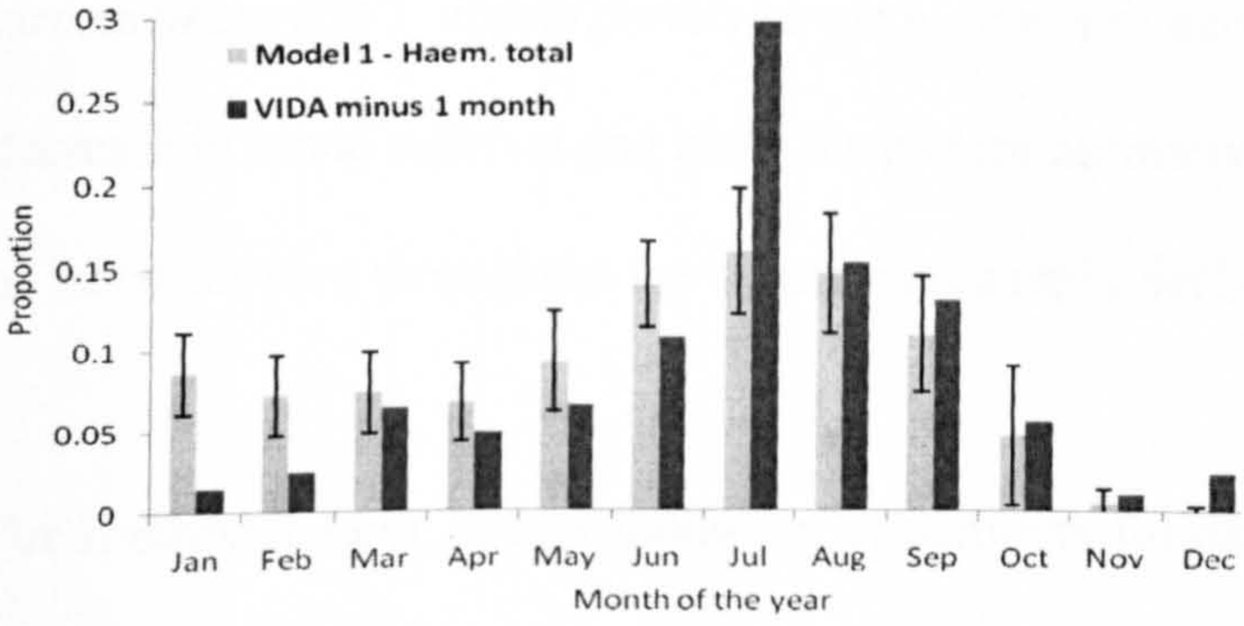
Predicted overall UK success increased significantly in the order *T. colubriformis* - *T. circumcincta* - *H.contortus* ($J = 8748$, $z = 13.453$, $p < 0.001$). The differences in predicted success between all three species were highly significant ($U \leq 0.001$, $z = -8.959$, $p < 0.0001$).



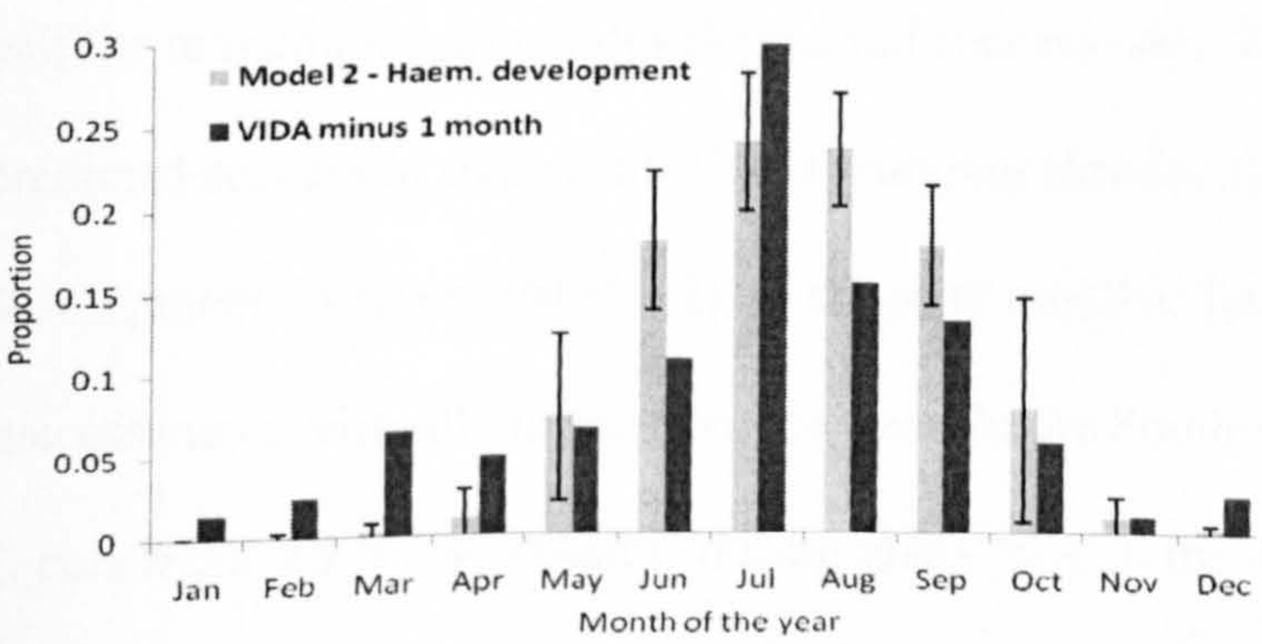
A. NOS- Model 1



B. NOS- Model 2



C. Haemonchosis – Model 1



D. Haemonchosis – Model 2

Fig. 3.5 Predicted and actual (VIDA) relative contributions of the months of the year to parasite success; In the NOS category disease outbreaks lag predicted success by two months, in the category Haemonchosis the time lag is one month; Proportion = the monthly contribution to the total of annual predicted success or outbreaks; Error bars represent 95% confidence intervals.

3.5.2 Sensitivity analysis

Influence of μ_1 on predicted success of development and survival models

For all three species, the influence of increasing μ_1 is illustrated for the weather station Paisley (fig. 3.6). Predicted egg development- related success (Model 2) is only sensitive to changes in larval death rates at higher temperatures during the 'peak season'. Small increases of laboratory-derived μ_1 values have a relatively great influence on developmental success while further increases (i.e. above Mu 3) have a decreasing impact.

This also holds true for over-winter survival-related parasite success (Model 3). When run on laboratory-derived larval death rates ('Mu 1') the model predicts significant proportions of *T. circumcincta* and *T. colubriformis* larvae to live well into the next year. Mu 3 shows a dramatic decrease in larval survival and gives the pattern normally observed at pasture, where high levels of larvae survive throughout the winter with a rapid decline throughout the spring.

For *T. circumcincta*, large windows of opportunity for development exist during the winter months, even in Scotland, while the predicted success of these windows runs at approximately half the maximum summer developmental success rate. The species also shows high levels of predicted success of over-winter larval survival (Model 3), running at rates very similar to developmental success (Model 2) in the peak months. Together the two give a 'flat' total daily success curve, virtually throughout the year. In the Southwest (not shown here) development of *T. colubriformis* is also possible during many days in the winter months. However, as

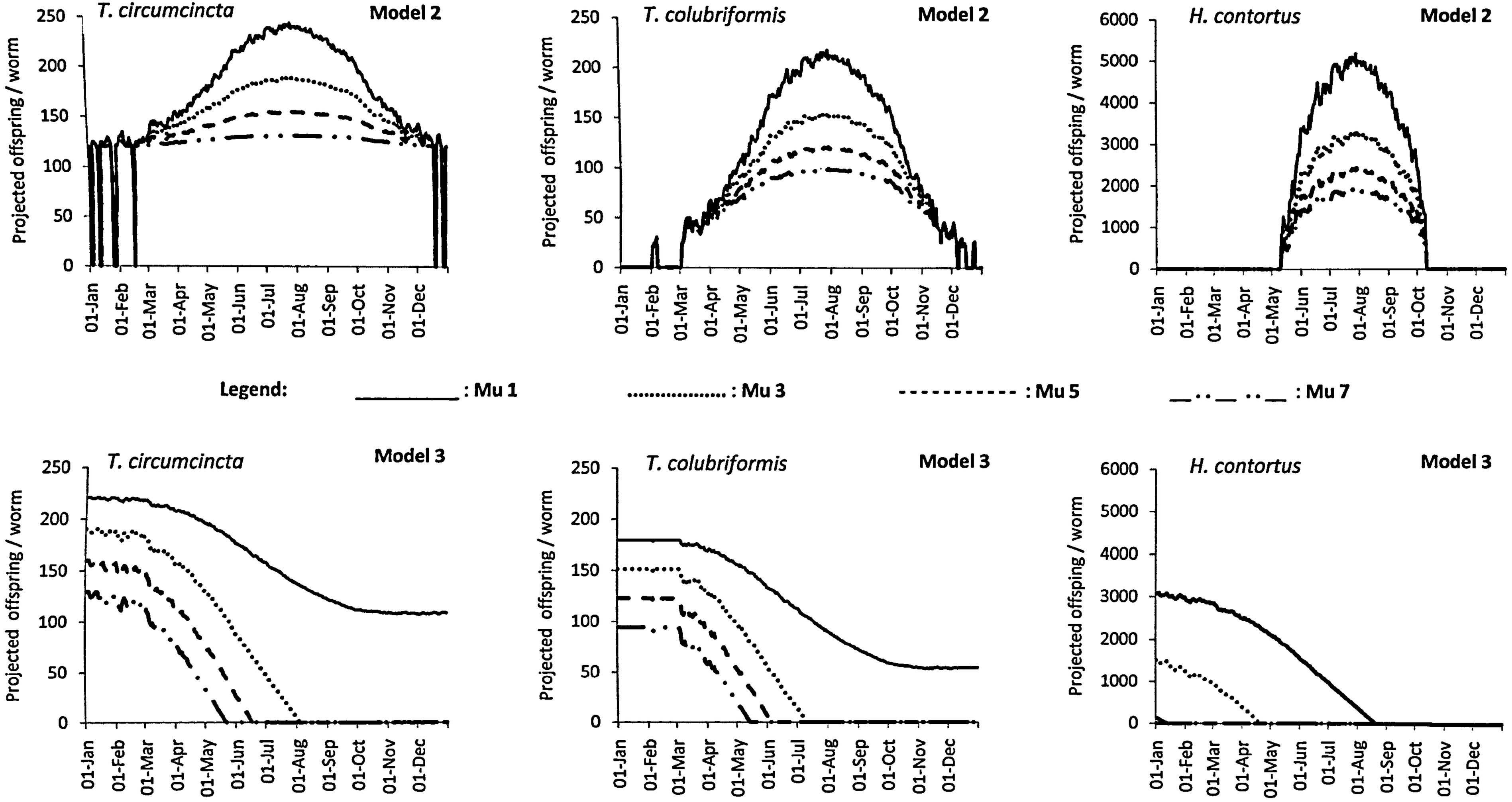


Fig. 3.6 The influence of larval death rates on the projected offspring per worm. Model 2 represents the projected success based on the development of current eggs only (equation 3.4); Model 3 gives the projected offspring of infective stages carried over from the previous year (equation 3.5).
The models were run on mean daily mean 1977-2006 temperatures for Paisley (Scotland).

temperatures only just exceed the developmental temperature threshold, the success rate of these developmental spells is approximately one tenth of that of the peak summer months. Developmental windows for *H.contortus* do occur in the Southwest in winter, but very inconsistently. Also, maximum success rates related to larval survival (Model 3) are approximately half those the result of egg development (Model 2), while the larval survival of this species is the most sensitive to increases in μ_1.

Whatever the multiplication rate of μ_1 the potential developmental success of eggs, for *T. circumcincta*, already runs at high levels before the numbers of surviving larvae start to decline rapidly. For *T. colubriformis* development takes place at this moment in time but runs at very low levels while for *H. contortus* there is a two-month discrepancy between the rapid decline of larvae at pasture and the start of egg development.

The results of the Mann-Whitney U tests on differences in the predicted success of larval survival between 'Scotland' and 'the Southwest' are given in table 3.7. At Mu 1 level no significant differences are found between the two regions for any of the three species but at Mu 3 the level of predicted success from over-winter survival becomes very significantly better in Scotland for all three species.

	T. circumcincta	*T. colubriformis*	*H. contortus*
Mu 1	U =343, p =0.228	U = 370, p = 0.432	U = 310, p = 0.086
Mu 3	U = 162, p < 0.001	U = 193, p < 0.001	U = 163, p < 0.001
	r = -0.747	r = -0.657	r = -0.744

Table 3.7 Test statistics of Mann-Whitney U tests comparing predicted parasitic success ascribed to over winter survival of larvae in Scotland and the Southwest. Mu 1 represents laboratory-derived μ_1 values, Mu 3 the model run on three times these values.
The Bonferroni-adjusted significance level is 0.008.

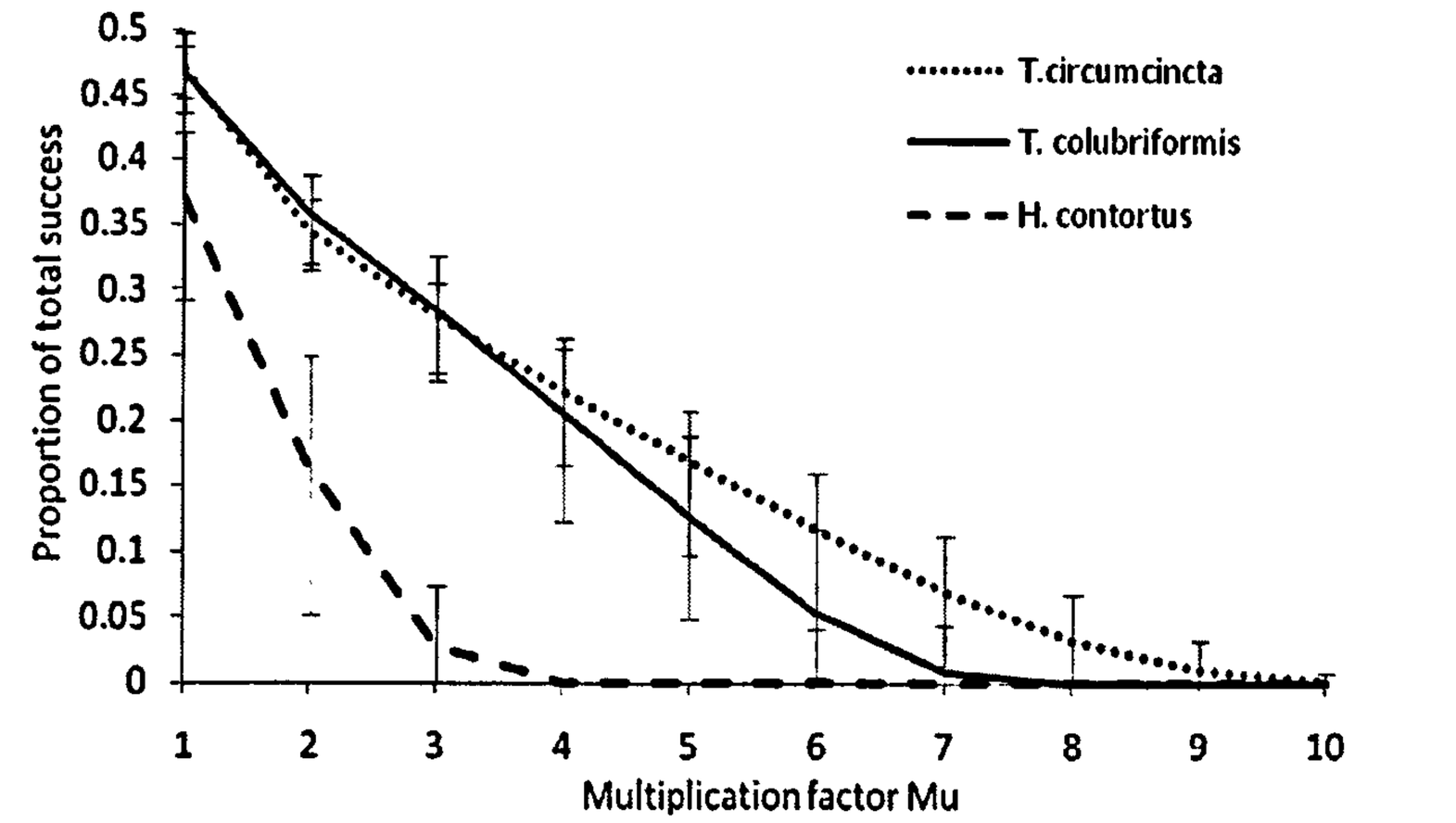
Influence of μ_1 on dynamics

For all three species, at any level of μ_1 multiplication, the potential for contribution of 'survival' (Model 3) to the total parasitic success (Model 1) is predicted to be greater in Scotland than in the Southwest but, as the 95% confidence intervals overlap, not significantly. For both regions, the contribution of surviving larvae to total Q_0 declines rapidly with increases in μ_1, (as is illustrated for the Southwest in fig 3.7 A.), i.e. Model 3 is more sensitive to μ_1 than Model 2.

Whatever the value of μ_1, larval survival (Model 3) contributes significantly less to the total offspring for *Haemonchus* than for the other two species (Fig 3.7 A.). The total annual predicted offspring of *H.contortus* is approximately ten times that of the other two species (Fig 3.7 B).

As expected from the above, the total predicted success is sensitive to two to five-fold increases of μ_1 but not sensitive at all to further increases (fig 3.7 B). Ten-fold increases of μ_1 result in an approximately three-fold reduction of *T. circumcincta*, four-fold reduction of *T. colubriformis* and six-fold reduction in *H. contortus* offspring.

A.



B.

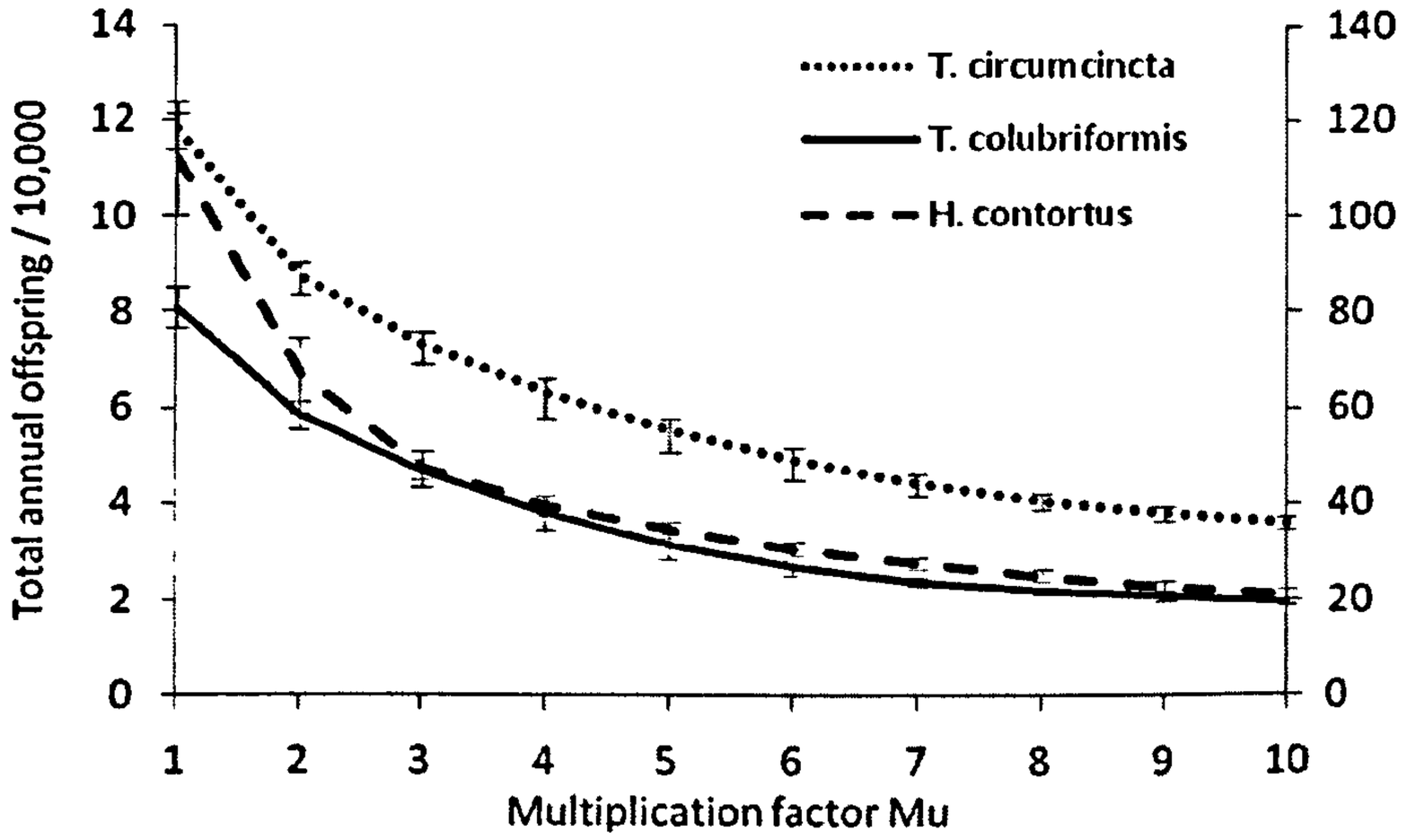
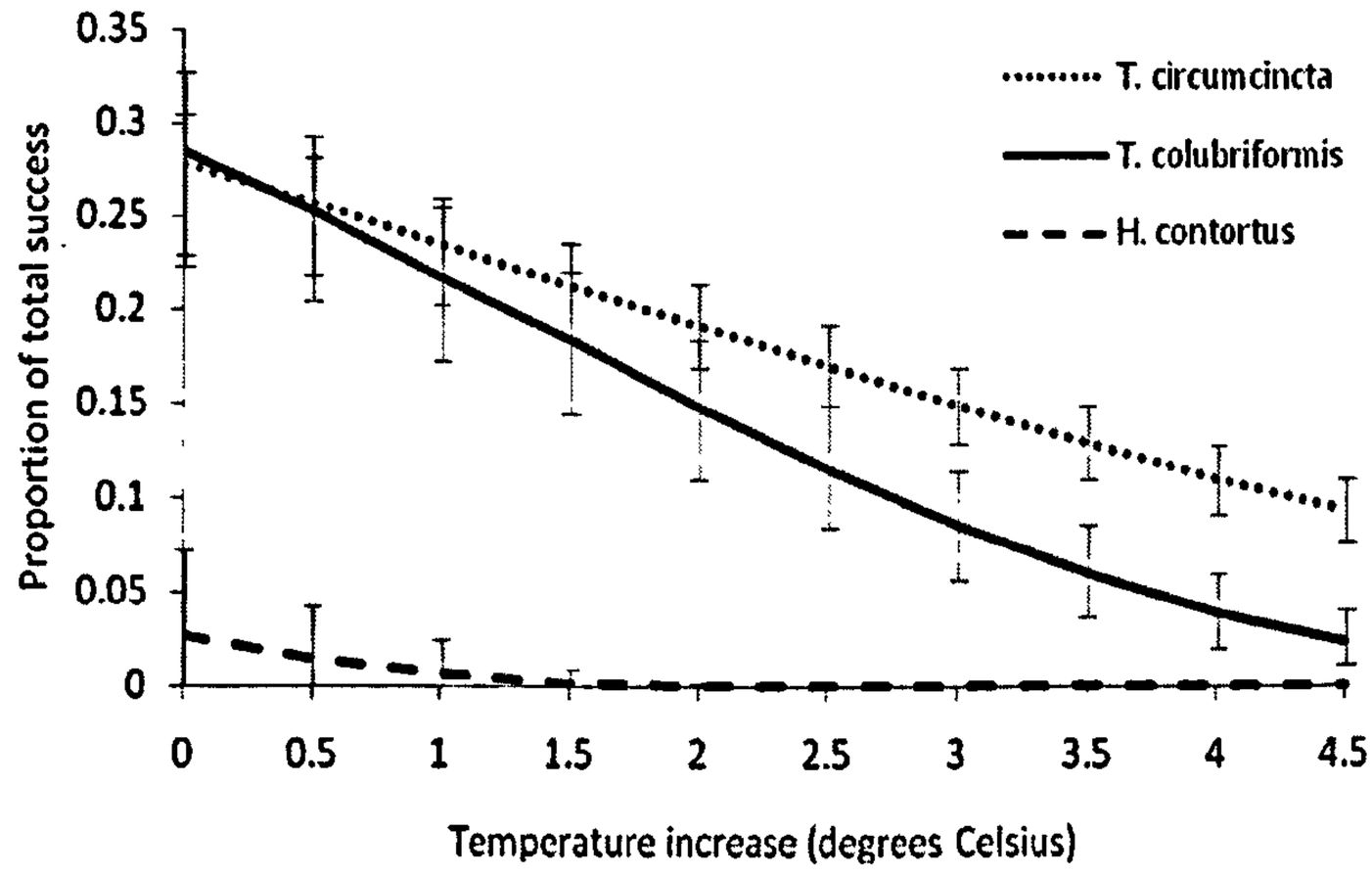


Fig. 3.7 The influence of increases in μ_1 on the proportional contribution of $Q_{0(s)}$ to total Q_0 (Model 3 relative to Model 1; **A.**) and the total Q_0 for one year x 10^{-4} (Model 1; **B.**) in the Southwest; Error bars represent 95% CIs; In Fig. B., the right vertical axis (multiplication factor 10) applies to *Haemonchus contortus* only.

3.5.3 Climate change

For all three species the model predicts that further increases in mean daily temperatures will decrease the relative importance of larval over-winter survival for parasitic success (see fig. 3.8 A.). Alterations in the total mean offspring per worm show a very consistent pattern for both regions (see fig 3.8 B). *T. circumcincta* is predicted to remain at similar levels while very small increases in *T. colubriformis* transmission may be observed. Total *H. contortus* offspring per worm, however, is predicted to increase in a linear or even exponential manner.

A.



B.

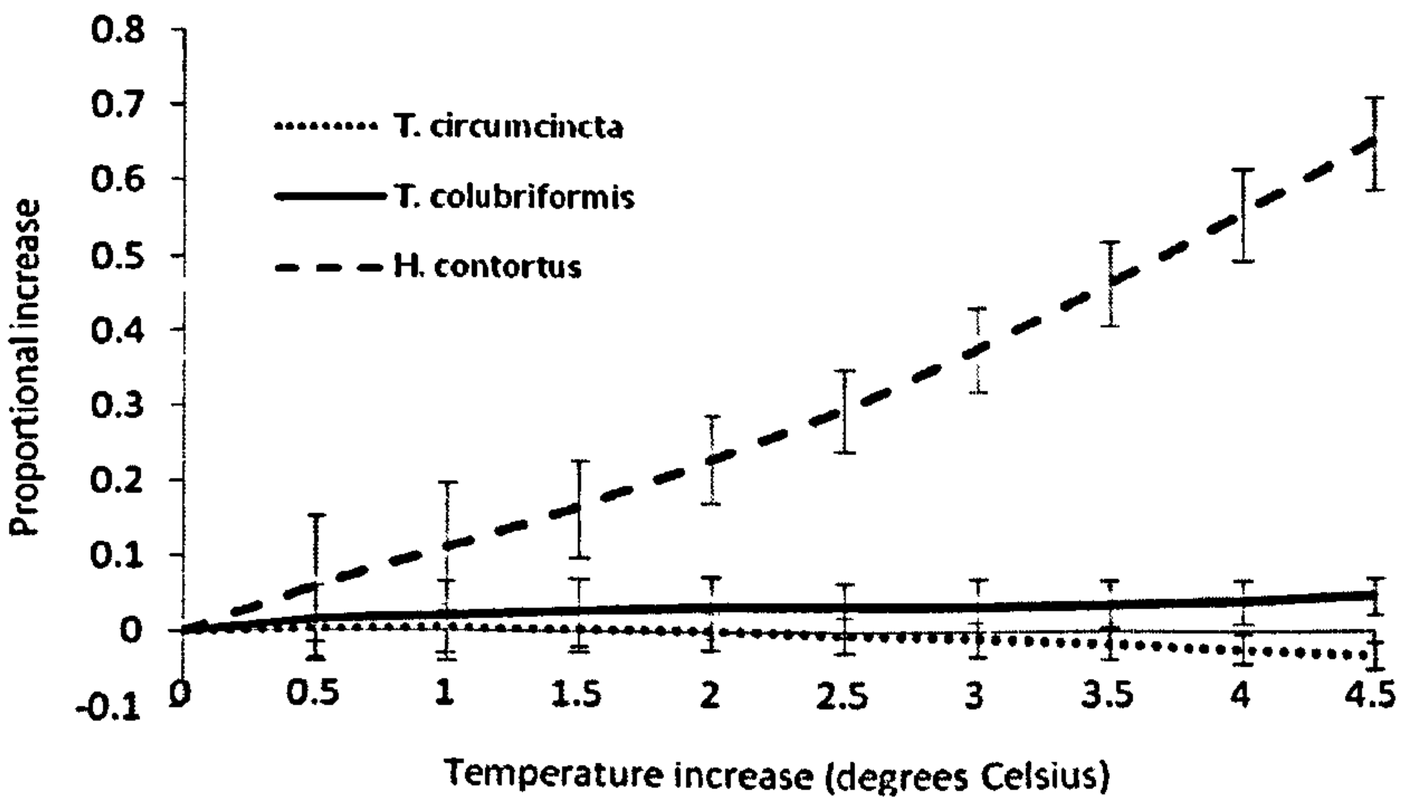


Fig. 3.8 The influence of temperature increases on the relative contribution of larval over winter survival to the total of parasite offspring (Model 3 relative to Model 1; **A.**) and total predicted offspring, relative to the 1977-2006 situation (Model 1; **B.**). In fig B the point zero represents the 1977-2006 mean and the Y axis the predicted increase relative to this point.

Fig 3.9 gives an insight into why this may occur. For *Haemonchus contortus,* regardless of the value of μ_1, the benefits of extra development outstrips the disadvantages of increased larval death throughout the year. For *T. circumcincta* and *T. colubriformis*, the benefits of extra thermal energy are, in certain months, more than offset by negative effects on larval survival. For these two species, at the more realistic μ_1 rates (for example Mu 3; see fig. 3.6), the model reflects the patterns observed in the clinical disease data presented in the previous chapter: during the spring reproductive success is predicted to decline while during the summer and autumn increases may be observed. Fig. 3.9 also shows that small alterations in larval death rates, apart from having a large effect on the total predicted annual parasitic success (Fig. 3.6), may cause temporal shifts in success of parasites. For example, a change from Mu1 to Mu3 reverses trends predicted for the spring.

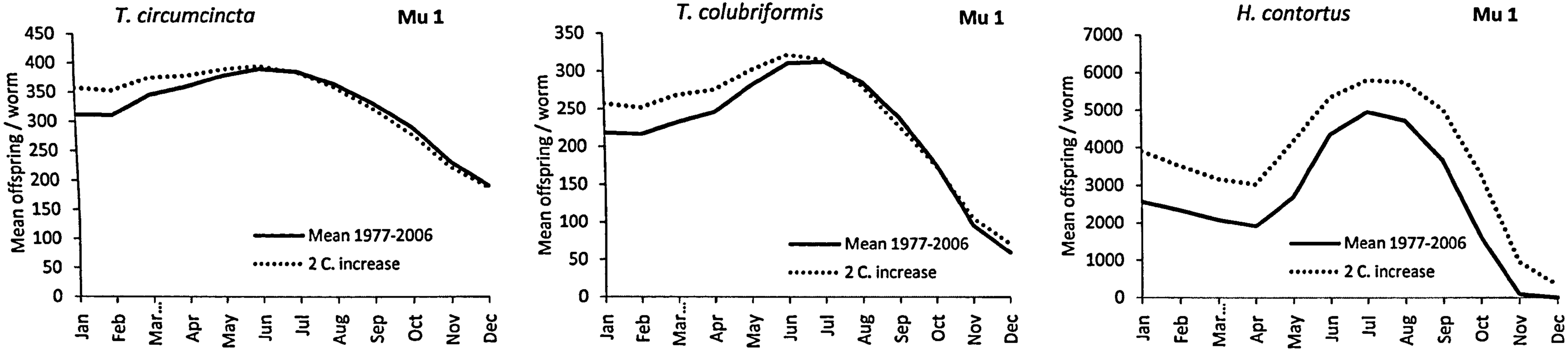


Fig. 3.9 The influence of temperature increases, and μ_1, on predicted offspring of worms over the months of the year. Mean daily 1977-2006 mean temperatures were modelled as well as year-round daily 2˚C increases. The scales of the vertical axes differ for each graph.

3.6 Discussion

The basic reproduction quotient model by Kao *et al.* (2000) was developed as a tool to compare the relative between-species success of species, and differences in success between countries. This model, which described parasitic success the result of development in a certain year only, was extended to include the potential of parasitic offspring resulting from larvae which had developed at pasture in the autumn of the previous year, and was run on mean daily temperature data. It appears that such a simple daily-transmission potential tracking model, incorporating temperature as the sole climatic variable, captures the dynamics of the studied parasites very well. The model not only reproduced larval abundance patterns observed at pasture but also produced strong correlations with disease incidence data. It captured observed (chapter 2) regional differences in parasitic success while simple temperature increase scenarios reproduced described shifts in the relative importance of certain months for parasitic success (chapter 2). It appears that, currently, temperature is the single most important driver behind the abundance of trichostrongyloids and that all other climatic factors have relatively little influence. Also, mean daily temperature alone appears to be a good predictor of seasonal trends in larval abundance of those trichostrongyloids completing transmission on the completion of the development phase (i.e. all but those delaying the release of infective larvae in the environment, such as *Nematodirus* spp.).

All three species are predicted to thrive in the UK. The Q_0 values given here are very much higher than those calculated by Kao *et al.* (2000). This, apart from the higher UK stocking densities used here, is the result of the fact that migration of larvae has not been included in the

model. Only a larval proportion of approximately 0.02 is likely to make it onto herbage (see chapter 6). Also, total Q_0, in this chapter, is the sum of output of two different worms (one producing eggs before winter and one at the modeled time point) while Kao et al. model the predicted offspring of one worm. If these factors are taken into account the Q_0 values are quite close to those given by Kao *et al.* Chapter 6 will address the question whether a model neglecting migration can actually describe larval patterns of emergence accurately.

For the species in the category NOS (*T. circumcincta* and *T. colubriformis*) the development model (Model 2) gave the strongest correlation with disease incidence data while output from the survival model (Model 3) tended to have lower values with higher disease rates. This suggests that either disease normally occurs after infection with larvae that have developed in the same calendar year and/or that the negative effects of warmer years on larval survival are more than compensated for by increased development potential. Model 1, including both egg development and larval over-winter survival, did not predict the spread of outbreaks over the months of the year significantly better than the development model, again suggesting that over winter survival at pasture, although it may be important in the year-to-year persistence of the species, plays a minor role in terms of disease risk in the spring. Especially in recent years, both in the Southwest and in parts of Scotland, development of *T. circumcincta* appears to be possible throughout the year with a relatively high level of predicted offspring in the winter. For *T. colubriformis*, winter development has, in recent years, increased in potential, but does not as yet appear to be a reliable phenomenon. Also, as development rates at lower temperatures are lower than those of *T. circumcincta*, the predicted winter development success of the species is rather insignificant when compared to the summer potential. These differences in development rates during the

colder months of the year between the two species closely reflect the findings of the detailed pasture studies by Gibson and Everett (1967, 1972).

Over the past 29 years, for *Haemonchus contortus*, apart from development-related Q_0 (Model 2), survival-related Q_0 (Model 3) was also positively correlated with diagnostic rates. Running the model on individual years showed that, in the warmer winters, pasture survival of the species was significantly better. This would suggest that over-wintering larvae at pasture, which would ensure larval uptake at a time when it is still too cold for larval development, could be involved in a lengthening of the *Haemonchus* season and a more intense build-up of pasture contamination. However, the model including larval survival (Model 1) did not give a significantly better fit of month-to-month VIDA data than the development model (Model 2) alone and the correlation between predicted survival success and disease incidence may just be an artifact of warmer years. The predicted *Haemonchus* success preceded the VIDA data by just one month suggesting that the build-up of L3 to dangerous levels, courtesy of the large λ of the species, may take place within one or two worm generations and that an earlier start to the *Haemonchus* season may not necessarily lead to more disease outbreaks.

Even if the build-up of worm burdens over several generations is taken into account by the application of certain time lags, both the 'NOS' and *Haemonchus* models, when compared with disease abundance data, tend to overestimate disease in the months directly preceding the peak but underestimate the actual peak month. This is likely to illustrate important limitation of these types of models: if predicted success of parasites is high disease may be more likely but whether it occurs depends on host fitness and immunity. The model does not include a clinical threshold for disease while animals will only get sick if burdens rise above this threshold.

Hudson *et al.* (2006) modeled the effect of temperature increases on host-parasite dynamics of systems with free-roaming hosts. They predicted that, for species to which the host acquired strong immunity during infection, the effects of climate change would not translate to an increased disease incidence. Very young lambs may not be able to mount strong immune responses to *Haemonchus contortus* (McClure *et al.* 1998; Smith and Angus, 1980) but are able to control species like *T. colubriformis* (McClure *et al.* 1998). Lambs approximately six months of age have been shown to expel large adult burdens of *Haemonchus contortus* (Barger et al. 1985; Smith and Angus, 1980). The presented correlation between predicted parasite abundance and disease incidence, and increased disease incidence in months that only older sheep are present at pasture, may indicate that host immunity is not able to nullify an increased force of infection in artificially intensified host-parasite systems.

The development threshold of *T. circumcincta* , together with the relatively high proportion of eggs developing at lower temperatures, appears to be very well suited to the UK. Together they ensure that, at the moment the numbers of larvae surviving at pasture start a steep decline, the predicted success from fresh eggs shed at pasture is already at very high levels. This would ensure a fairly consistent level of larval presence at pasture throughout the year. The more all-year-round, flat curve, nature of disease outbreaks in Scotland (chapter 2) may be the result of the species being relatively more abundant in the colder regions.

T. colubriformis development of eggs also starts when the probability of development of over-wintered larvae declines but, at that moment in time, only at a level of approximately one third of the predicted success resulting from survival.

For *H. contortus*, apart from larval over winter survival being a far less constant between-year phenomenon, there is an at least two-month discrepancy between the start of the rapid decline in over wintered larvae and the start of the development season. Based on this, the degree to which hypobiosis is recruited by the different species for year-to-year survival would be expected to be highest in *Haemonchus contortus*, lower in *T. colubriformis* and lowest in *T. circumcincta*.

Unfortunately, no studies investigating hypobiosis have included all three species. In the temperate regions, it has been shown that, at the same location, much larger proportions of *H. contortus* larvae than *T. circumcincta* inhibit development in the autumn (Thomas and Waller, 1979; Waller *et al.* 2004). A climate-related inhibition of trichostrongyloid development has traditionally been viewed as a function of larval survival (e.g. Eysker, 1993). However, the proportion of a species inhibiting, and the length of the period of inhibition, may also be driven by the minimum temperature threshold for development. Indeed, it may just be that the 'peakedness' of the development phase, which is determined by the value of the minimum development threshold, determines both the onset of hypobiosis in the autumn and the timing of the resumption of development in spring. This would mean that the 'peaky' appearance of *Haemonchus* at pasture would be the driver, and not the result, of hypobiosis. Although this may appear to be a trivial point it is important for our predictions on the evolution of hypobiosis in relation to climate change. As the result of warming the model predicts larvae to survive for a shorter period of time. If larval survival were the main driver of hypobiosis it could be hypothesized that the proportion of larvae inhibiting in the autumn would increase as a result. However, if hypobiosis is seen as a function of the minimum development threshold, although larval survival would decrease in importance, the season during which larval development could take place would increase accordingly. In this scenario, only the length of the period of inhibition

would be optimised. Once the temperature remains above the development threshold all non-inhibiting coding genomes would be favoured. Non-inhibiting *Haemonchus* isolates have been described in Kenya (Waruiru *et al.* 2001). Future models attempting to predict the longer- term effects of climate change on parasitism will explicitly need to include parasite evolution.

The contribution of over winter survival to the total of annual offspring is small and therefore it could be expected that larval survival does not play an important role in the epidemiology of the three species species. However, the total annual Q_0 appears to be very sensitive to small increases in larval death rates. Increasing larval death rates to above literature rates was also shown to alter predicted spatial (1) and temporal (2) trends:

1) At laboratory-derived $\mu_1$values, although, as the result of higher temperatures, spring-larval survival is shorter in the Southwest than in Scotland, the total of predicted survival Q_0 does not significantly differ between the two regions. At these low-level Mu values, increased death rates in the spring can be compensated for by higher proportions of development in the warmer autumn of the previous year. However, if Mu is increased to Mu 3, the benefit of the warmer autumn is more than outstripped by the higher death rates in the spring, and larval survival is significantly better in Scotland. The larger relative importance of larval survival in Scotland may be a second explanation for the more year-round disease patterns in Scotland described in chapter 2. It is interesting to note that *Haemonchus* survival is predicted to contribute more to Q_0 in Scotland than in the Southwest. It has been assumed that the winters, at UK latitudes, are too cold for *Haemonchus* (e.g.Troell *et al.* 2005). However, as long as ground frosts do not reduce the temperature lower than a few degrees Celsius below zero during the winter, given the energy reserves of the species, it appears that spring temperatures are more limiting. Also, the

developmental temperatures in the previous spring are suboptimal for this species and therefore relatively lower numbers enter the winter.

2) With regards to climate change, for *T. circumcincta* and *T. colubriformis*, increasing Mu 1 to Mu 3 gives a reverse of predicted spring trends. Reduced parasitic success in spring would be explained as under 1). These predictions explain the spring patterns described for the NOS category in chapter 2 and confirm that currently experienced warming is already likely to change parasite epidemiology.

Summing of temperature-dependent contributions of individual days to the probability of one cohort of larvae dying as the result of depletion of energy reserves, at Mu values of 3 and above, reproduced realistic larval survival characteristics (fig. 3.2.6). Two British studies (Gibson and Everett, 1967; Boag and Thomas, 1970) described how levels of live *T. colubriformis*, overwintering on grass plots, remained high until the start of April and then fell rapidly during spring to zero in or around the month of June. Boag and Thomas (1970) included *T. circumcincta* in their study and described how *T. colubriformis* died off more rapidly in spring. This is also evident from Gibson and Everett (1972), a study showing *T. circumcincta* numbers start their decline in April but fall to zero only in July, August or even September.
The Mu 3 *Haemonchus* model predicts the last larvae to die off towards the end of April, exactly in the way described by Gibson and Everett (1976). Grenfell *et al.* (1986) investigated differences in mortality rates of infective *Ostertagia ostertagi* in water and on herbage. From their data it can be extracted that the instantaneous daily mortality rate of larvae at pasture was approximately 3 times as high as that of larvae kept in water at 20°C. The model presented here

sums daily mortality rates rather than multiplying survival rates (Grenfell *et al.* 1986), but nevertheless supports multiplication of daily mortality rates by this magnitude at pasture. As factors such as desiccation are also thought to be determinants of larval survival it is surprising that a multiplication of laboratory-derived temperature-related larval death rates with a constant describes patterns of larval die-off so well. However, as confounding climatic factors limiting larval abundance may be correlated to temperature it cannot be concluded that temperature determines larval abundance at pasture.

In terms of global warming, in the short term, disease resulting from *T. circumcincta* and *T. colubriformis* infections is predicted to have a more 'peaky' appearance, and more cases are likely to be seen later in the year. This may implicate lower worm burdens for very young lambs but higher infection levels of older animals. The immuno-epidemiology of infections in older animals has received very little research attention and it is currently unclear 1) to what extent older animals will show clinical signs of disease, and 2) to what extent they contribute to pasture contamination.

The *Haemonchus* season is predicted to be extended both into spring and autumn. Whether increased predicted success in spring will lead to alterations in disease patterns will depend on how hypobiosis will be adapted to climate change. It is likely that increased developmental success during the summer will lead to more disease. Increased success in the autumn is likely to lead to higher burdens of arrested L4-stage larvae in older animals and this may in turn ,once again, lead to increased levels of pasture contamination in spring. This may well lead to disease, and death, in ewes as well as lambs (Sargison *et al.* 2007; van Dijk and Morgan, 2006). If larger numbers of parasites over winter in their host, as more parasites will be in contact with

anthelmintics during treatments, the rate of development of drug resistance in this species is likely to increase (Martin *et al.* 1981).

Lastly, the model predicts future species shifts. It is predicted that further warming may benefit trichostrongylus species but not the arctic-adapted *T. circumcincta*. The predicted benefits to *Haemonchus contortus* are alarming and it is likely that more disease will be witnessed in the future. To what extent this will happen is not clear. Firstly, rainfall patterns are also predicted to be altered and *Haemonchus* is relatively sensitive to desiccation (chapter 1). Secondly, at current temperatures, *Haemonchus* is already predicted to be the most successful species in the UK but this is not reflected in the diagnostic rate, which is much lower than for the species in the NOS category. It appears *H. contortus* is as yet not as ubiquitous on UK farms as the other commonly diagnosed species. However, as many farmers are still not familiar with the clinical signs, the presence of the species may also be very much under diagnosed.

3.7 Conclusions

A simple, daily-mean-temperature driven model of parasitic success incorporates enough complexity to explain all the epidemiological trends uncovered in chapter 2. The model confirms that climate change is likely to already have had an effect on parasite epidemiology.

Periods of risk of disease to animals appear to be strongly correlated to windows of opportunity for larval development. Small differences in minimum development threshold between species appear to result in large differences in epidemiology, and the threshold may, to a large extent, determine the way species will adapt to future climate change. Perhaps surprisingly, for *T.*

circumcincta and *T. colubriformis*, larval death rates determine the periods of maximum risk within these windows and are also crucial for the predictions of responses of these parasites to future climate change. Mathematical models of parasite transmission should therefore always incorporate larval death rates derived from pasture data. As these death rates are likely to be under influence of factors other than temperature, the study of the effects of climatic change on larval death rates is one area that should be prioritized for future research. The evolution of hypobiosis in response to environmental factors, and the infection dynamics of these nematodes in older, partially immune, animals, are also areas that urgently need to be addressed. The ecology and epidemiology of *Haemonchus contortus*, a species making extensive use of the host for over-winter survival, appear to be best suited to future temperature increases. Farms currently not contaminated with this species should prioritize the species in biosecurity protocols.

As even the most basic parameters and thresholds of the species *Nematodirus battus* have not been published, it is currently not possible to qualify, let alone quantify, responses in the abundance of this species to climatic influences. The next chapter makes a start on addressing this void.

Chapter 4 - The influence of temperature on the development, hatching and survival of *Nematodirus battus* larvae

4.1 Introduction

The species *Nematodirus battus* Crofton and Thomas 1951, a member of the subfamily Nematodirinae (Nematoda: Trichostrongyloidea), was discovered relatively recently. Especially after the first UK nematodirosis *battus* outbreaks in the 1940's and 1950's the effects of the synchronised hatching of infective larvae on young lambs were very much feared by sheep farmers. From VIDA data (chapter 2) it would be estimated that, in the 21st Century, the parasite still kills a few thousand lambs annually in the UK alone. In affected flocks morbidity and mortality often run at high rates (typically 50 -100%, and 5 - 20%, respectively; Kingsbury, 1953; Thomas and Stevens, 1956) with substantial impact on animal welfare and farm economics. The data presented in chapter 2 suggest that the parasite is far from under control.

In a large phylogenetic study Hoberg (2005) was able to show that the Nematodirinae originated in the Holarctic with primary distributions determined across Beringia. Some of the species of the genus *Nematodirus* Ransom, 1907, have since been introduced all over the world. The free-living stages are well adapted to extreme environments, being relatively resistant to low temperatures and desiccation (Hoberg, 2005), and the genus has become established in a wide variety of climates. *Nematodirus battus* has so far mostly been confined to the temperate regions of the northern hemisphere. The species was first

discovered in Great Britain in 1951 (Crofton and Thomas, 1951) and has since been recorded in Norway (Helle, 1969), the Netherlands (Borgsteede *et al.* 1978), Canada (Lichtenfels *et al.* 1997), the USA (Hoberg *et al.* 1986), Germany (Bauer, 1989), Denmark (Thamsborg *et al.* 1996a), Poland (Fudalewicz-Niemczyk *et al.* 1996), and Sweden (Lindqvist *et al.* 2001). The mass emergence of third-stage larvae (L3) from embryonated eggs in spring is thought to occur only when a period of prolonged exposure to low temperatures (close to zero Celsius) is followed by a rise in temperature to above 10 °C (Thomas and Stevens, 1960; Christie, 1962; Parkin, 1972) and such conditions occur in all of the these countries. However, *Nematodirus battus* has also been shown to persist in sheep in Mexico (Sanchez and Quiroz Romero, 1993) and was recently described as a 'dominating species' on Sicily, Italy (Torina *et al.* 2004), even though the mean minimum winter temperature rarely drops below 11 °C on this Mediterranean island. This raises the question of how far south this economically important parasite could extend its transmission range, and also what climatic conditions are needed for it to become economically damaging. The study of arctic parasites which have moved to warmer regions may in itself provide us with models for future adaptation to global climate change. The ecology of arctic parasites has been shown to be very sensitive to climatic changes (Kutz *et al.* 2005; Jenkins *et al.* 2006b).

The sparse amount of research conducted on the free living stages of *Nematodirus battus* is in sharp contrast to its great importance as a disease of livestock. Following its discovery some laboratory and field studies were carried out (e.g. Christie, 1962; Gibson and Everett, 1981), but the behaviour of the parasite at pasture remained insufficiently understood. For example, the existence of a second, autumn, peak in larval emergence was described (e.g.

Rickard *et al.* 1987) but it is not clear what drives the hatching of these eggs. Here we describe laboratory experiments to determine the vital rates and thresholds that underpin the hatching behaviour of *Nematodirus battus*, with a view to (i) be able to parameterise mathematical models of the field ecology of the parasite, which can assist with the development of control strategies; (ii) determine the potential for an extension of the current global transmission range to warmer climates; and (iii) predict how this originally sub-arctic parasite will respond to climate change in the regions where it is currently established.

4.2 Materials and methods

4.2.1 Egg recovery and larval development

As no pure culture of eggs was available, fresh dung from sheep naturally infected with *N. battus* was collected at a farm near Bristol, UK. It was mixed with at least 10 parts tap water to one part dung in a mechanical blender. The slurry was passed across a course sieve of 2.0 mm aperture and then a sieve of 35 µm aperture. In order to remove more roughage, the egg-containing residue on the fine screen was first submitted to 60% sucrose and then 16% NaCl flotation. Remaining roughage was removed by pouring the watery suspension over a flat glass dish and leaving it to stand for 5 minutes, after which the eggs stuck to the bottom while the roughage could be decanted off (van Wyk, J.A., personal communication). The eggs were collected in filtered tap water and kept in Petri dishes for the development experiments. Faeces were collected from lambs during spring, when trichostrongylid nematodes are present at very low levels, and *Nematodirus battus* eggs, which are readily

distinguished from those of other species, comprised the vast majority of those collected. Virtually all of the few trichostrongylid eggs initially present amongst the *Nematodirus* eggs were lost during the egg recovery protocol and only three trichostrongylid L3, once again readily distinguishable from *Nematodirus battus* on tail length alone, were ever seen in wells during the following experiments.

Three replica Petri dishes, each containing approximately 4000 eggs, were placed in cooled incubators (Sanyo-Gallenkamp, UK) at 11, 11.5, 13, 15, 20, 25 and 30°C, and at 14-20°C (12-hourly fluctuation). Temperature was recorded at 10-minute intervals using TinytagPlus® data loggers (Gemini Data Loggers, UK). Each week 200 eggs from each Petri dish were examined in a 1 ml nematode counting slide (Chalex Corporation, USA), under 100x magnification. The proportion of eggs showing development and the proportion of eggs containing larvae were recorded separately, after which the eggs were discarded. If larvae were present in the eggs approximately 100 larvae were freed from the eggs by crushing them between two microscope slides. A drop of Lugol's iodine solution was added and the proportion of larvae that had developed to the larger, sheathed L3 stage was recorded. After the first L3 were found, eggs were examined every three days. Each time eggs were examined the Petri dishes were screened for the presence of hatched larvae under a stereo microscope.

All data were analyzed using SPSS statistical software, release 12.0 (SPSS Inc., USA). Correlations between development rates and temperature were assessed by Pearson correlation and linear regression. The developmental threshold temperature was calculated

by extrapolating the regression line of daily developmental rate against temperature to the point at which no development was predicted to occur. The number of Degree Days taken for 50% of the population to develop to L3 was calculated by deducting the predicted minimum development threshold from the measured temperatures and multiplying by the number of days taken to 50% development. These simple techniques are commonly used in entomology (e.g.Williams and Richardson, 1984; Wall *et al.* 1992; Ames and Turner, 2003) and have been successfully applied to nematodes (e.g. Samson and Holmes, 1985; Kutz *et al.* 2001). Levene's test was used to test for homogeneity of variance between treatments. The regression model for time to 50% development (D_{50}) as a function of temperature was used to predict the D_{50} of the 12-hourly fluctuating 14.5-20°C treatment. The distribution of the D_{50} found in the experiments was assumed to differ significantly from this predicted value if the means were more than 1.96 Standard Deviations apart. The observed time between the discovery of the first L3 and the point at which all eggs that were to produce L3 had done so ($t_{min\text{-}max}$) at each temperature, and the arcsine transformed percentages of eggs completing development at each temperature, were analysed using one-way Analysis of Variance (ANOVA) and Tukey's pairwise comparison.

4.2.2 Egg survival at higher temperatures

Unembryonated eggs were kept at 30°C for 4 weeks, then placed at 15°C and their development followed as above. The survival of embryonated eggs at higher temperatures was examined by allowing L3 to develop to the pre-hatch stage at 25°C, then comparing the proportion of L3 emerging from eggs immediately subjected to temperatures suitable for

hatching, with those kept at 25°C for a further 4 weeks. In each case hatching was stimulated by 5 weeks of chilling at 4°C followed by an increase in temperature to 13°C. Treatments were compared using t-tests on time taken for 50% of eggs to develop, and on arcsine-transformed proportions of eggs hatching.

4.2.3Hatching

The influence of periods of chilling on the magnitude and timing of the hatch at 15°C

Fresh eggs were kept in a watery suspension at 20°C for 7 weeks, at which time all developing eggs had progressed to the L3 stage. After mixing, the suspension was split into two aliquots, one half remaining at 20°C and the other half chilled at a constant 4°C. At 2, 3, 4, 6 and 12 weeks, 3 replicates of approximately 500 eggs were pipetted into 96-well microtitre plates for each treatment, with 2-10 eggs per well in 120 µL of water. The number of viable eggs and the number of larvae, if present, were counted under a stereo microscope. The plates were sealed with Parafilm® (Pechiney Plastic Packaging, USA) to prevent desiccation and placed at a hatching temperature of 15°C. The number of larvae present in the wells was again counted on day one and from then onwards every other day. The plates were followed until no further hatching was observed in three consecutive counts. At each count the plates were screened and wells topped up with filtered water as necessary. In order to assess whether eggs not hatching in the non-chill treatment were actually still viable, and whether they would indeed only hatch after a period of chilling, the eggs of the 4, 6 and 12 week non-chill treatment were, after the initial hatch had finished, placed at 4°C for 4, 6 and

12 weeks, respectively, then put back at 15°C and followed as before. The arcsine-transformed proportion of eggs hatching and the time taken for 50% of the eggs to hatch (t_{50}), were analyzed by one-way and two-way analyses of variance (ANOVA) and Tukey's pairwise comparison.

Temperature thresholds for hatching

Eggs were developed to the L3 stage and split into chilled and non-chilled aliquots as above. Three replicate plates of chilled and non-chilled eggs were then placed at hatching temperatures of 6, 9, 11, 13, 15, 17 and 20°C. The number of larvae present in the wells was counted on days 7, 10, 13, 15, 20 and then every ten days, until no further hatching was observed for two consecutive counts. In order to assess the effect of temperatures fluctuating beyond the hatching range, one batch of eggs containing L3 was placed at 4°C for three months, then pipetted into wells as above and 9 replicates were placed at a hatching temperature of 15°C. On day 10, at which time hatching had commenced, three replicates were placed at 6°C and three replicates were placed at 20°C. On day 16 these were put back with the other plates, at 15°C. Data were analysed as above.

4.2.4 Larval survival

Larvae that had hatched at 13°C from chilled eggs were divided into Petri dishes and placed at temperatures of -5, 6, 11, 13, 15, 17, 20, 25 and 30°C as well as 12-hourly 14-20°C fluctuation and 6°C with one weekly 15-hour 'night-frost' of -5 °C. To determine whether

chilling of eggs affects larval survival, larvae that had hatched at 13°C from eggs that had not undergone any chilling treatment were put at 11, 13, 15, 17 and 12-hourly 14-20°C fluctuations. Two replicates were used for each treatment. Every 10 days, up to day 140 (approximately the length of one summer or winter), 200 larvae from each dish were examined in a 1 ml nematode slide and the number of L3 alive counted. Larvae were assumed to be dead when immobile and in a characteristic stretched out position, and were discarded after counting. Mortality rate was estimated as the slope of the linear regression of $\log_{10}$-transformed proportion of larvae alive against time. Differences between treatments were considered significant when the 95% confidence intervals of the slope parameter did not overlap. Time to 50% survival (L_{50}) and the predicted proportion of larvae alive at 100 days (P_{100}) were calculated from the regression equations. The 100-day survival indicator was chosen because during relatively warm arctic summers the temperature stays above 10°C for approximately 100 days (Kutz *et al.* 2002).

4.2.5 Egg development and hatching at pasture

After adding a small amount of water, dung from naturally infected sheep was thoroughly mixed in a mechanical blender. The number of eggs per gram in the mixture was assessed by performing McMaster egg counts on 15 random samples of 3 grams. On 07-07-2005, four heaps of 140 grams of mixture, each containing 35,700 eggs (bootstrap 95% CI 33,416-37,984) were placed in the centre of pasture plots 1x1 meter in size. Eggs were also freed from dung as described above and incubated in water at 20°C. On 30-09-2005, after 7 weeks of incubation, these non-chilled embryonated eggs were divided into 4 aliquots of 20,000

(bootstrap 95% CI 18,968-21,032) in 20 ml of water and evenly spread out over the central 50x50 cm of 1x1 meter plots. Soil temperature in the upper 1.5 cm of soil, between the grass roots, and rainfall were recorded by a Skye MiniMet weather station (Skye Instruments Ltd., UK). From 08-09-2005 onwards, once a month, grass on all 8 plots was cut with scissors at a height of 1.5 cm and removed (i.e. sampled without replacement). The number of *N. battus* larvae present on the herbage was assessed by a, modified, sucrose-interface method first described by Eysker and Kooyman (1993). Grass cuts were weighed, then soaked in 16 liters of tap water for 18 hours. The grass was then thoroughly washed, and the water squeezed out, twice. It was put on trays, in an oven, at 80°C for 8 hours or until thoroughly dry, then weighed again, making a calculation of L3 recovered per kg dry matter herbage possible. The larvae-containing water was poured over a sieve with an aperture of 1mm and the pieces of grass remaining on the screen added to the trays in the oven. The water was twice poured over a sieve with an aperture of 35 µm and the material on the screens transferred to 250 ml, funnel shaped, beakers and left to stand for 1 hour. The supernatant was carefully siphoned off and the sediment transferred to 50 ml Falcon tubes. The 250 ml beakers were washed twice with small amounts of water which was added to the Falcon tubes. The Falcon tubes were spun at 2000 rpm for two minutes and the top 35 mls siphoned off (i.e. leaving sediment and water amounting to 15 mls in total). The remaining sediment was thoroughly mixed with the remaining water. 20 mls of a 60% Sucrose solution was now injected under the water/sediment mixture, with a blunted needle attached to a syringe, taking care not to mix the sucrose and the suspension. The tubes were spun for 5 minutes at 2000 rpm, after which a clean blunted needle was introduced at the larvae-containing interface. 15 mls were syringed off, transferred to clean Falcon tubes and 15 mls of clean tap water was added. 6

drops of Lugol's solution were also added and the tubes were left to stand for five minutes. The number of *N. battus* larvae present in 1 ml was counted three times, making use of a rostered nematode counting slide (Chalex Corporation, USA) and the mean of three counts multiplied by 30 to give the total number of larvae recovered.

Sampling continued until June 2006. During January and February 2006 grass growth was too limited to sample the plots.

4.3 Results

4.3.1 Larval development

Results are shown in Table 4.1. Below 11.5°C eggs did not reach the L3 stage and at 30°C no development occurred at all. Linear extrapolation from the proportion of eggs developing at 15, 20 and 25°C predicted the upper development temperature to be 28°C. However, at higher temperatures faster development was offset by a reduction in the number of eggs developing such that towards the upper end of the temperature range the proportion of eggs reaching the L3 stage was greatly reduced. Differences in the proportion of eggs developing to L3 at different temperatures proved significant ($F_{5,17} = 167.8$, $p < 0.001$). There was no difference between the 11, 13, 15 and 14-20°C treatments (Tukey's $p \geq 0.297$), but the in the 20°C treatment significantly fewer eggs developed ($p < 0.001$) while at 25°C fewer eggs developed than at 20°C ($p < 0.001$). The optimum temperature for development was therefore estimated to be close to 15°C.

T_s	T_m	D_{50}	D_{max}	$t_{min-max}$	DD_{50}	% L3
11	10.9 (10.3-12.0)	Incomplete development	-	-	-	-
11.5	11.5 (10.9-12.5)	51 (50-51)	57 (55-58)	7 (6-9)	741 (731-746)	81 (78-83)
13	13.4 (12.4-14.0)	49 (49)	54 (52-55)	5 (3-6)	807 (807)	80 (77-83)
15	14.9 (13.9-16.3)	47 (46-47)	51 (49-52)	7 (7)	841 (829-847)	83 (79-85)
20	20.7 (19.6-22.4)	34 (33-34)	46 (44-47)	18 (16-19)	801 (786-809)	59 (58-61)
25	25.2 (24.6-25.7)	28 (27-28)	46 (43-49)	25 (22-28)	783 (764-792)	21 (20-24)
30	30.0 (29.1-30.7)	No development	-	-	-	-
14-20	14.6- 20.1 (14.2-14.9; 19.8-20.6)	32 (31-32)	35 (34-37)	7 (6-9)	644 (631-651)	79 (79-80)

Table 4.1 *N. battus* egg development at various temperatures. T_s = temperature setting; T_m = mean measured temperature; D_{50} = average day 50% of the eggs had completed their development; D_{max} = average day development of all eggs was completed; $t_{min-max}$ = time between the detection of the first L3 and the completion of development (in days); DD_{50} = average number of Degree Days to 50% development; % L3 = percentage of the eggs present at the start of the experiment completing development. Presented percentages and days all rounded up to the nearest integer. The ranges are given between brackets.

The daily development rate to 50% egg development increased significantly with temperature (Pearson r_p= 0.98, p < 0.001) and is given in figure 4.1. Extrapolation of the regression line predicted the minimum development threshold to be -3.01°C.

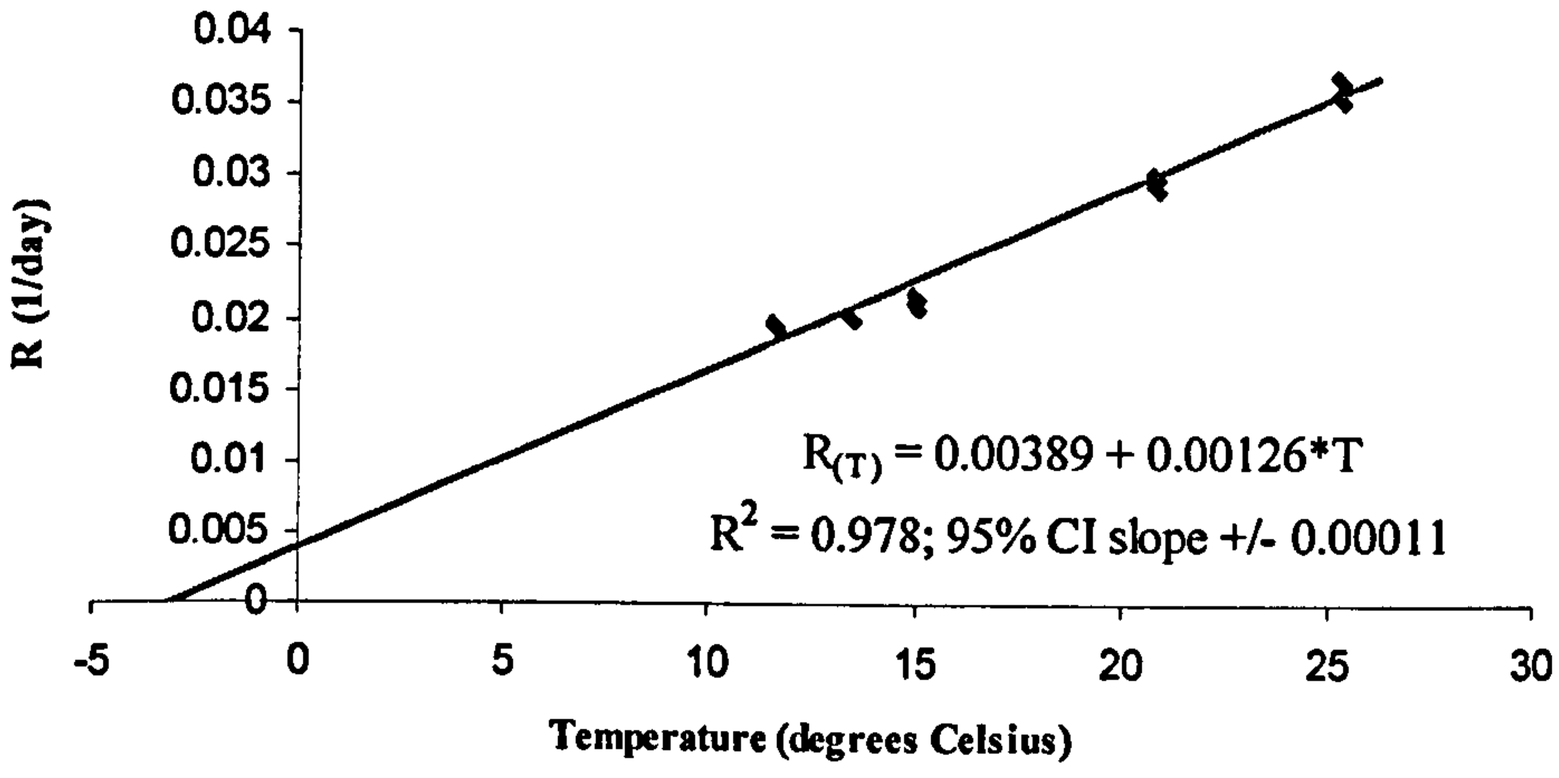


Fig. 4.1 Regression of daily development rate (R) on temperature. Please note that, as the result of overlap, not all 15 data points are visible.

The number of days to 50% development (D_{50}) decreased significantly with increasing temperature (r_p = -0.99, p < 0.001) and the regression line is described by D_{50} = 72.4 – 1.80*T (R^2 = 0.986, 95% CI slope +/- 0.128). This model predicts the D_{50} of the fluctuating 14.5-20°C treatment to be 41.3 days. The measured D_{50} of all treatments listed in table 4.1 showed homogeneity of variance (L = 0.20, p = 0.956) and the maximum within-treatment standard deviation was 0.57. Using the criterion that a separation of more than 1.96 times the standard deviation indicates a significant difference, development rate was increased by fluctuation in temperature.

The time taken between the onset and completion of development ($t_{min\text{-}max}$) differed significantly between treatments ($F_{5,17} = 56.14$, $p < 0.001$). There was no significant difference in $t_{min\text{-}max}$ at 11, 13, 15 and 14-20°C ($p \geq 0.775$), but at 20°C more time was taken to complete the development of all eggs ($p < 0.001$). At 25°C, $t_{min\text{-}max}$ was again significantly longer than at 20°C ($p = 0.006$).

4.3.2 Egg survival at higher temperatures

The time taken for development of L3 was not influenced by storage at 30°C ($t_2 = 0.71$, $p = 0.518$). However, the proportion of eggs that successfully hatched was reduced to 0.65 (from 0.83 at 15°C without exposure to higher temperatures; $t_2 = 9.4$, $p = 0.003$). The proportion of live L3 hatching was not significantly reduced by storage of embryonated eggs at 25°C ($t_2 = 0.99$, $p = 0.38$).

4.3.3 Hatching

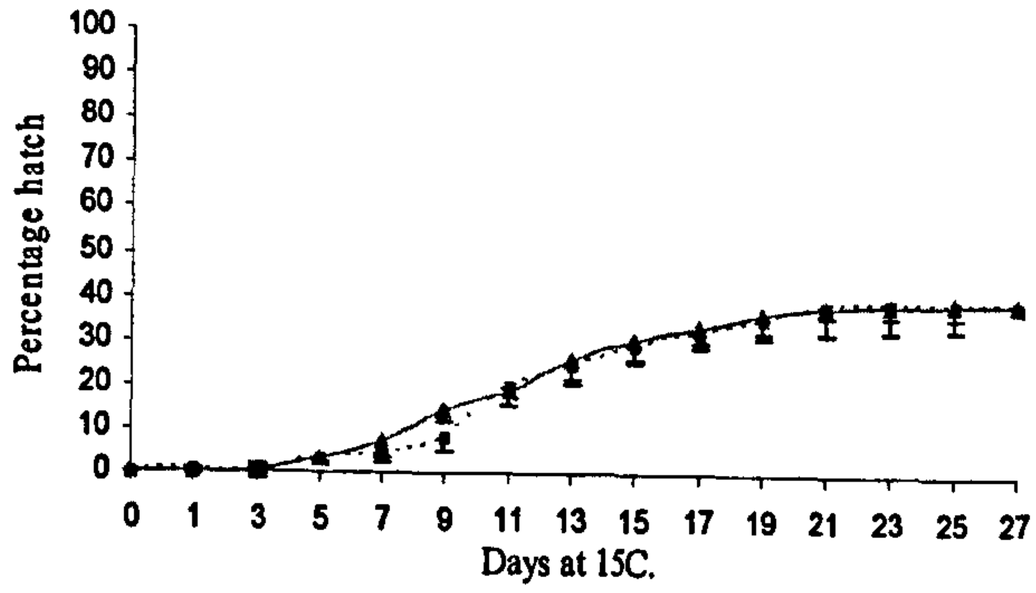
The influence of periods of chilling on the magnitude and timing of the hatch at 15°C

Hatching profiles of the chilled and non-chilled eggs is shown in figure 4.2. Overall the percentage of eggs hatching was significantly higher after chilling ($F_{1,29} = 432.9$, $p < 0.001$) and also increased significantly with the duration of chilling ($F_{4,29} = 26.1$, $p < 0.001$; interaction term $F_{4,29} = 110.3$, $p<0.001$). In relation to individual treatments there was no significant difference between the 2 and 3 week chill and the controls ($p = 0.98$), but chilling

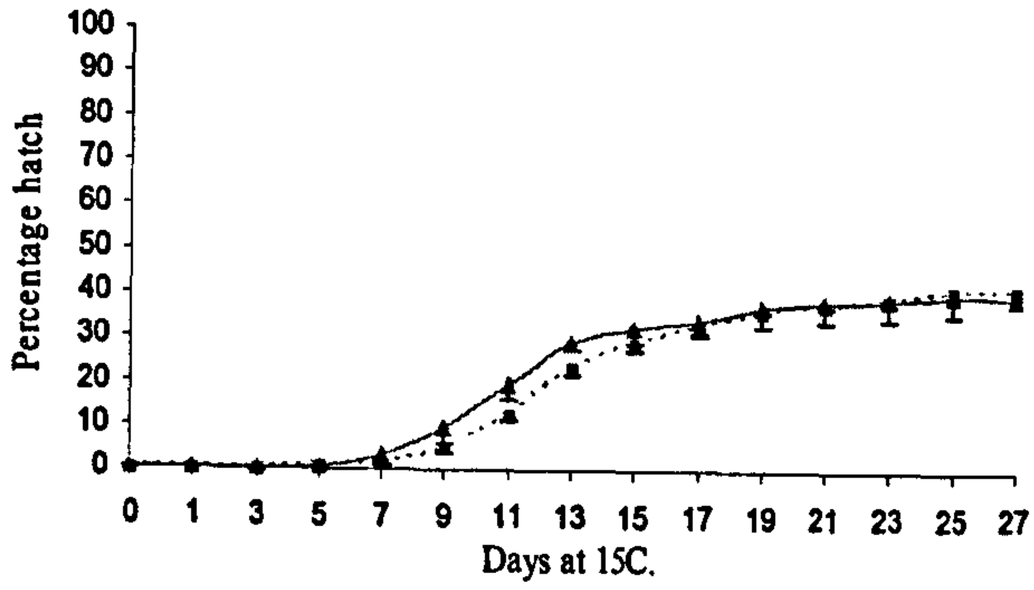
Legend:

..... : Chill treatments

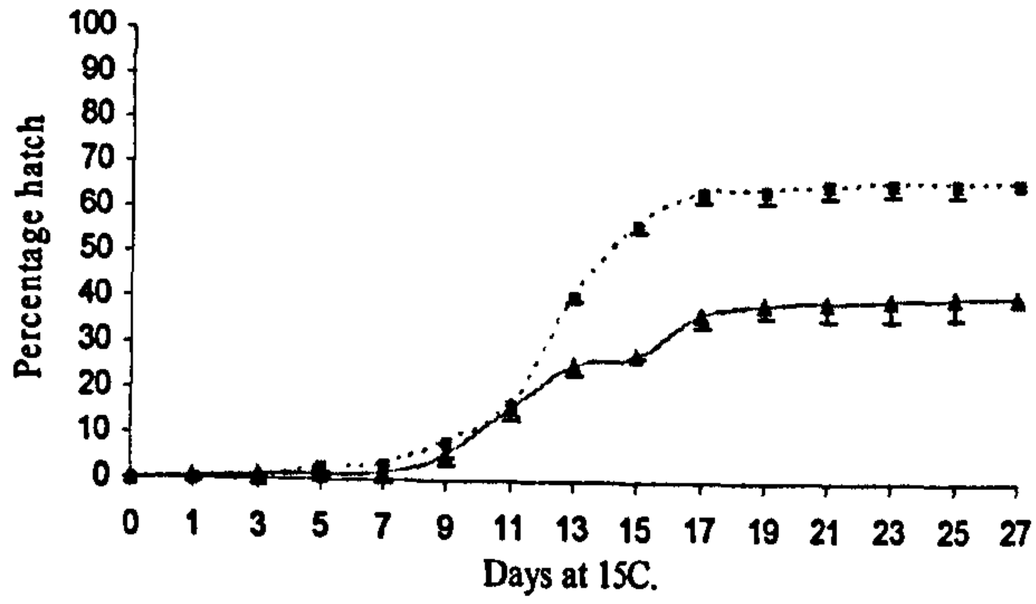
_____ : Controls



2 weeks



3 weeks

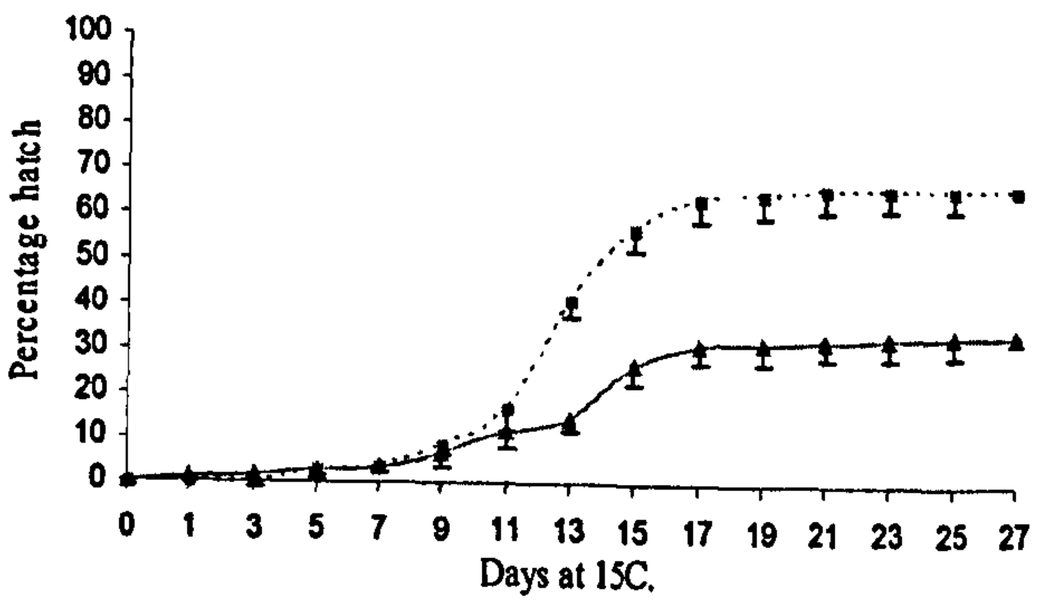


4 weeks

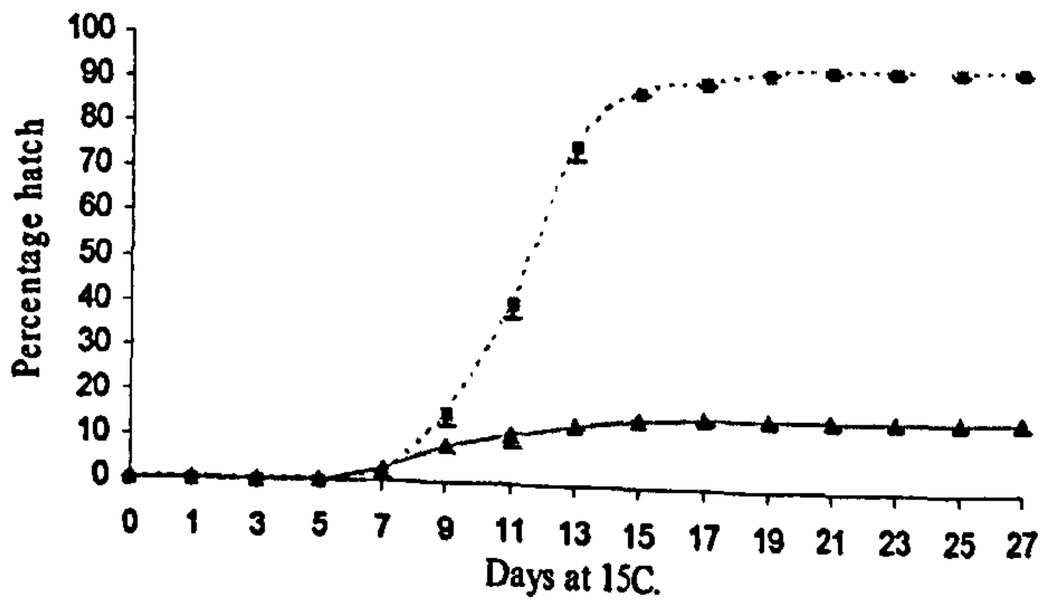
Fig. 4.2 The influence of 2, 3, 4, 6 and 12 weeks of chilling on the hatching of eggs at 15°C.

Eggs were chilled for the specified time at 4°C, or kept at a control temperature of 20°C, before each treatment was placed at 15°C for hatching.

Horizontal axis = the number of days spent at hatching temperature; vertical axis = cumulative percentage of eggs hatching. Error bars represent standard deviations.



6 weeks



12 weeks

for 4 or more weeks increased the proportion of eggs hatching ($p < 0.001$). Within the range of treatments where chilling had an effect there was no difference between 4 and 6 weeks of chilling ($p = 1.00$), but significantly more eggs hatched after 12 weeks of chilling ($p < 0.001$). In the non-chilled treatments the proportion of eggs hatching decreased after storage at 20°C ($F_{5,17} = 56,7$, $p < 0.001$). However, this decrease was only significant in the 12 week treatments ($p = 0.002$) and not in the 4 and 6 week treatments ($p = 0.070$). A large proportion of these eggs that did not initially hatch were stimulated to hatch after subsequent chilling. In the 4 and 6 week treatments, the total proportion of eggs hatching in this 2-phased hatch was not significantly different to that hatching after chilling alone ($t_2 = 2.6$, $p = 0.059$ and $t_2 = 2.7$, $p = 0.074$), suggesting that embryonated eggs can survive at least 6 weeks at 20°C without significant larval death. Although the proportion hatching after 12 weeks at 20°C followed by 12 weeks at 4°C was significantly reduced relative to chill alone ($t_2 = 7.8$, $p = 0.016$), live L3 were still recovered from 81% (range 80-82) of the eggs, compared with 92% (range 92-93) after chilling.

All 10 treatments completed 50% of hatching between the start of day 11 and the end of day 13. Although the variance of the t_{50} of the total of 30 replicates was very small (0.49), the 6 week treatments (both chill and non-chill) took significantly longer ($p = 0.011$) to hatch than the 2 and 12 week treatments ($F_{4,29} = 5.4$, $p = 0.004$). However, there was no significant difference in t_{50} between chill and had non-chill treatments ($F_{1,29} = 2.8$, $p = 0.111$; interaction term between weeks of treatment and chilling $F_{4,29} = 0.83$, $p = 0.52$).

Temperature thresholds for hatching

The proportion of eggs hatching in each treatment group, and their respective t_{50}, are given in table 4.2. In the treatments where hatching occurred, both lower temperatures and chill treatment significantly increased the percentage of eggs hatching ($F_{5, 35}$= 203.7, $p < 0.001$ and $F_{1,35}$=152.3, $p < 0.001$, respectively; interaction term $F_{5,35} = 24.3$, $p < 0.001$). Among the chill treatments hatching at steady temperatures, there was no difference in the proportion of eggs hatching at 11 or 13 °C, or at 15 or 17 °C, ($p \geq 0.89$). However, significantly more eggs hatched at 11 and 13 °C than at 15 and 17 °C ($p \leq 0.001$). In the non-chill treatments hatching at steady temperatures significantly fewer eggs hatched each time the temperature was raised by 2 °C ($p < 0.001$). Similarly, the eggs hatching without chill, expressed as a proportion of the egg population hatching after chill treatment, decreased with temperature ($F_{5,17}$= 136.3, $p < 0.001$), a smaller proportion of the egg population hatching without chilling at each 2 °C increase ($p \leq 0.04$). The magnitude of the hatch of both the chilled and non-chilled 11-15 °C and 14-20 °C fluctuating treatments did not differ from that at a constant 15 °C ($p \geq 0.80$).

Differences in t_{50} were highly significant between temperature treatments ($F_{5,35}$= 203.7, $p < 0.001$) and between chill and non-chill treatments ($F_{1,35}$= 152.3, $p < 0.001$; interaction term $F_{5,35}$= 24.3, $p < 0.001$). The timing of the chilled hatch was different from that of the non-chilled hatch at all temperatures ($p \leq 0.01$) except the 11-15 °C fluctuating treatment ($p = 0.06$). 13 °C was the only setting at which the t_{50} of the chill treatments was higher than that of the non-chill treatment. As shown in figure 4.3, hatching behaviour at 11°C without chilling

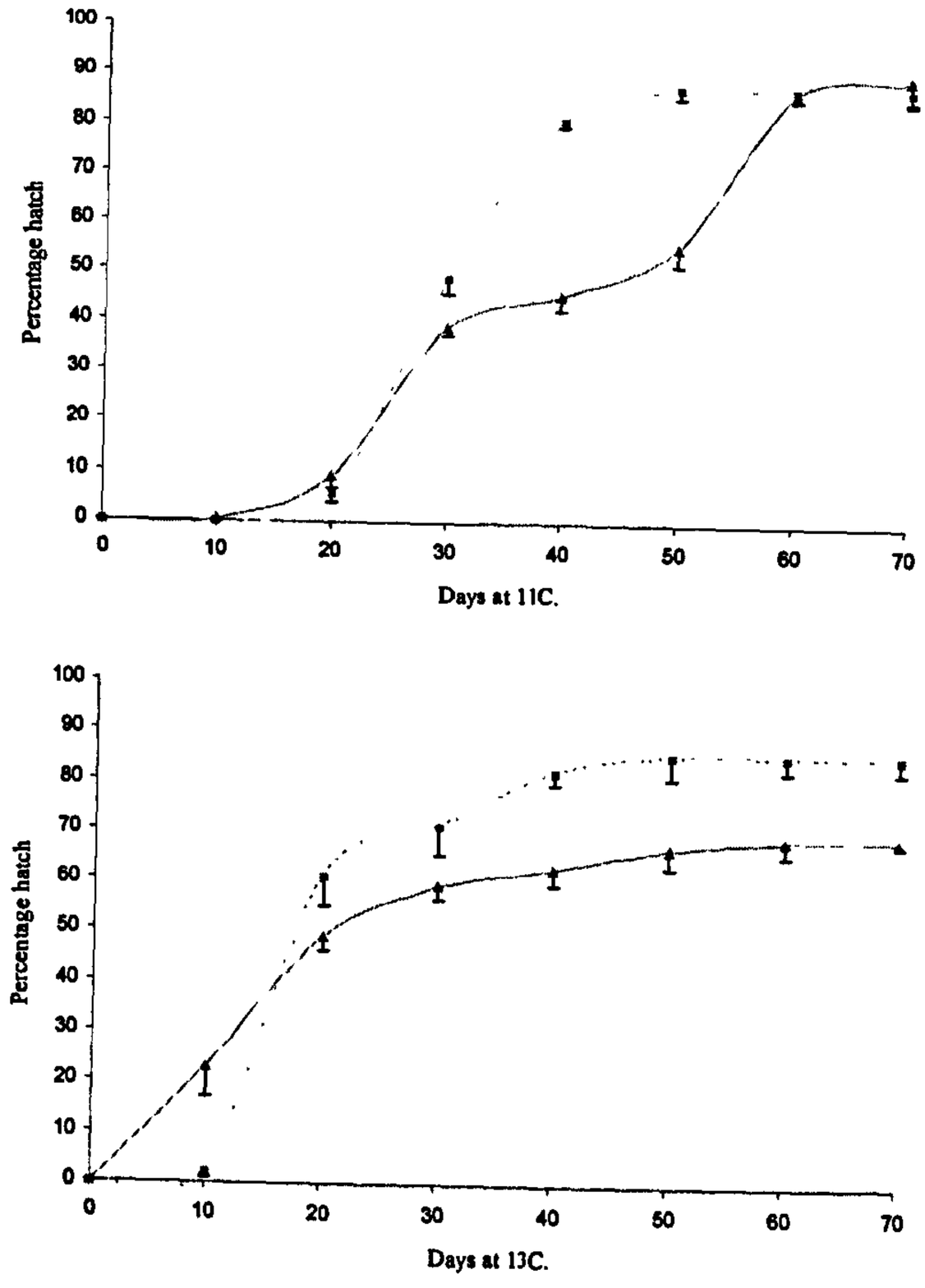
T_s	T_m	% Chill	% Non-chill	Proportion	t_{50} Chill	t_{50} Non-chill
6	6.0 (4.7-6.5)	< 1	< 1	-	-	-
9	8.9 (7.5-9.5)	< 1	< 1	-	-	-
11	11.0 (10.4-12.6)	86 (85-89	90 (84-93)	1	29 (28-29)	35 (32-38)
13	13.3 (12.3-14.1)	85 (82-90)	69 (68-70)	0.81	17 (17-18)	14 (12-14)
15	15.1 (13.9-16.3)	68 (64-70)	45 (38-52)	0.66	13 (12-13)	20 (19-20)
17	17.1 (16.5-18.3)	62 (59-67)	7 (5-8)	0.11	15 (15-16)	24 (24-25)
20	20.2 (19.8-21.1)	< 1	< 1	-	-	-
7-13	6.6- 13.4 (6.0-7.5; 12.1-14.0)	< 1	< 1	-	-	-
11-15	11.2- 15.6 (10.5-12.6; 15.0-16.7)	73 (70-74)	47 (41-53)	0.64	16 (16)	19 (19-20)
14-20	14.6- 20.4 (14.2-14.9; 19.7-20.9)	72 (71-73)	51 (46-57)	0.71	11 (10-11)	16 (15-19)

Table 4.2 Hatching of eggs at various temperatures. T_s = temperature setting; T_m = mean measured temperature; % chill and % non chill = mean percentage hatching in the chilling and non-chill treatments, respectively; Proportion = average of eggs hatching without chilling as a proportion of the average of eggs hatching after chilling; t_{50} Chill and t_{50} Non-chill = mean time (in days) to 50% hatch in the chill treatments and non-chill treatments, respectively. All measurements are rounded up to the nearest integer. Ranges are given between brackets.

Legend:

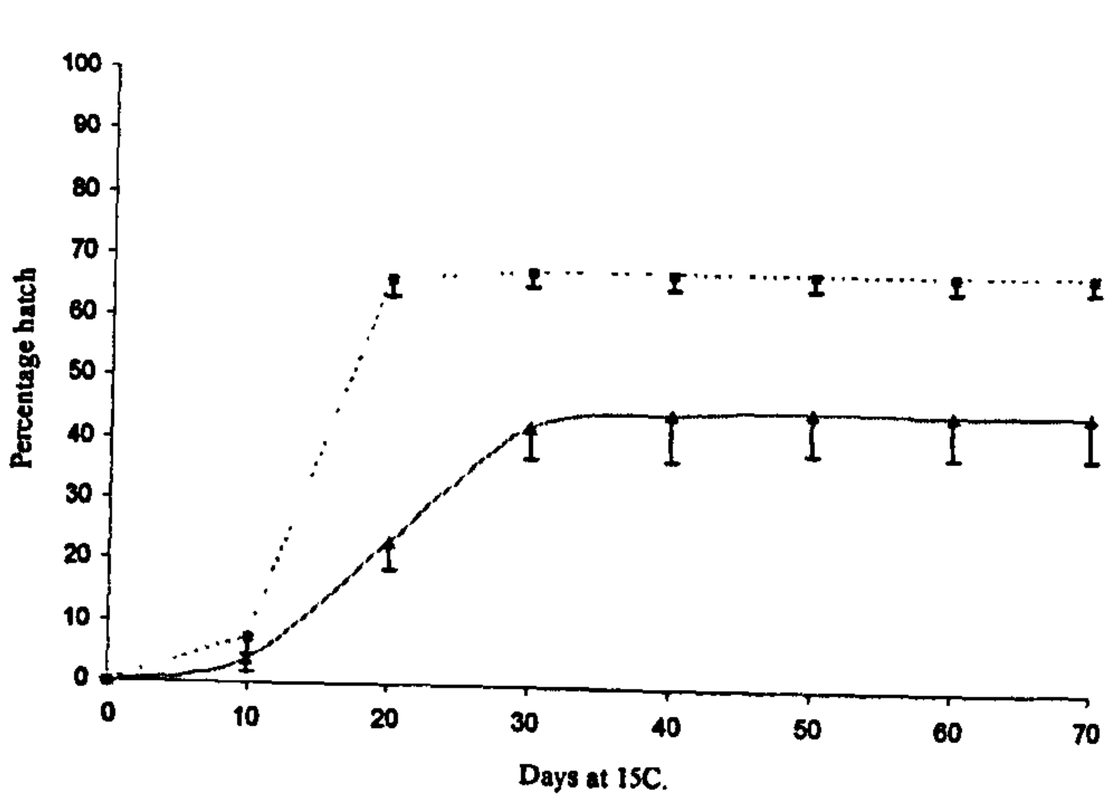
..... : Chill treatments

_____ : Controls



11°C

13°C



15°C

17°C

Fig. 4.3 Hatching of non-chilled and chilled eggs at varying temperatures.

On the horizontal axis the number of days at the given hatching temperature is given, on the vertical axis the cumulative percentages of eggs hatching. Error bars represent standard deviations.

was different from that at all other constant temperatures. The final proportion of eggs hatching at 11°C was higher than at other temperatures. However, 11°C initially proved a relatively weak stimulus for the hatching of chilled eggs, the maximum hatch only being reached after approximately 40 days. Hatching of non-chilled eggs at 11°C reached a plateau around day 30, after which little hatching occurred over the following 20 days, while the peak hatch was reached on day 60. Comparison of the shapes of these hatching patterns strongly suggests that a large proportion of the non-chilled eggs completed a chilling process at 11°C before proceeding onto hatching.

Interrupted hatching

The effect of fluctuations outside the temperature range for hatching is shown in figure 4.4. When hatching eggs were placed at 20°C the hatching process halted abruptly and resumed equally abruptly when the eggs were placed back at 15°C. Similarly, eggs put at 6°C halted their hatching process but it took a day or two longer for this to take effect.

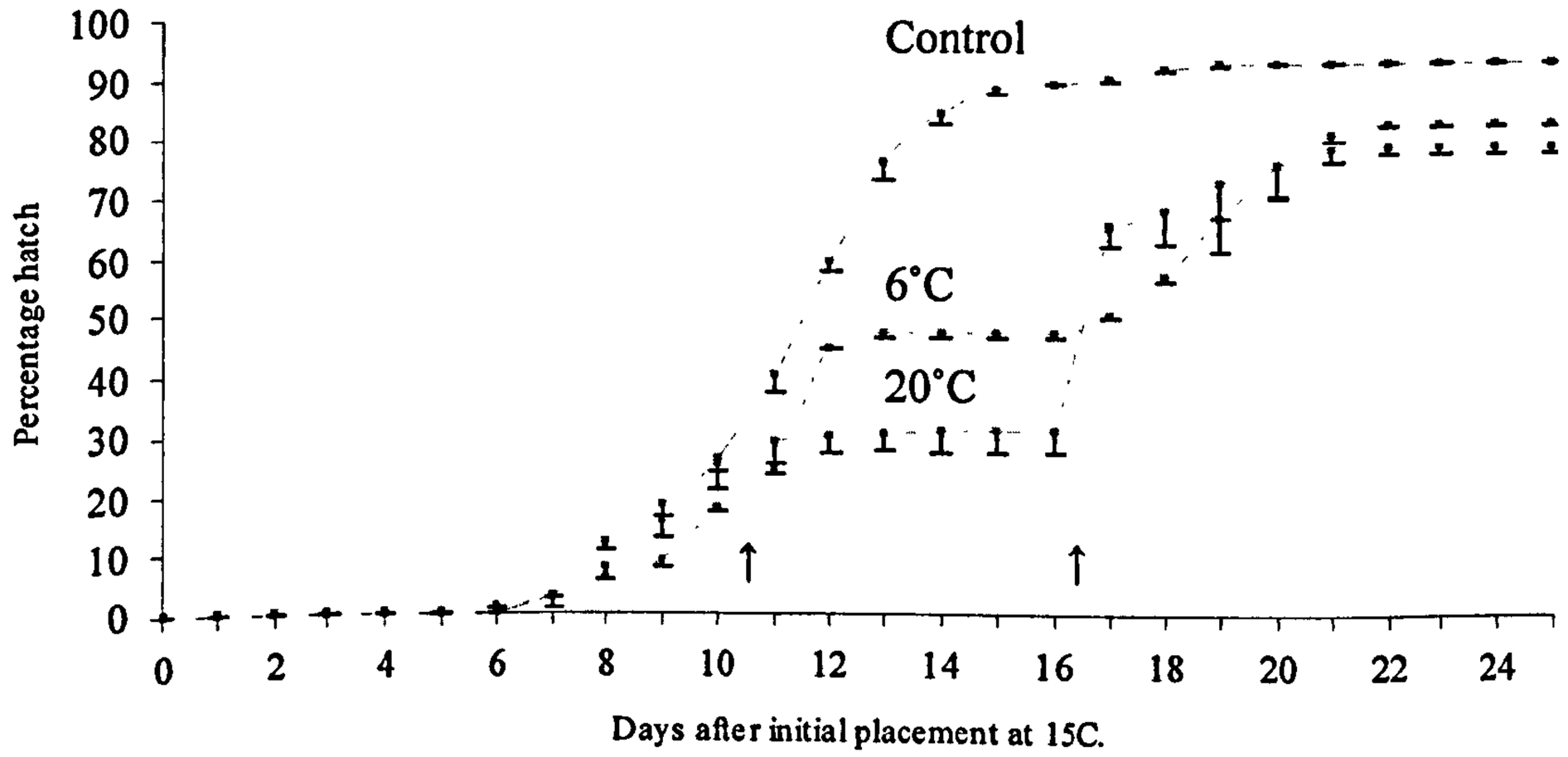


Fig. 4.4 The influence of temperatures outside of the hatching range on the hatching process;. The control was kept at 15°C continuously but treatments 6°C and 20°C were placed at these temperatures on day 10 of the hatching process (first arrow) and back at 15°C on day 16 (second arrow); Error bars represent standard deviations.

4.3.4 Larval survival

Chilled larvae

Log_{10} of surviving L3 was highly significantly correlated with time for all treatments ($p \leq 0.002$). Survival characteristics are given in table 4.3. At -5°C and at 6°C+ frost, survival was significantly better than at 6°C. Mortality rate did not differ at 6, 11, 13 and 15°C. At 17°C, however, larval survival decreased. At fluctuating 14-20°C larvae appeared hyperactive and died significantly quicker than at a constant 17 °C. Using the criterion of non-overlapping confidence intervals of the regression slope, mortality rate increased significantly at each 5°C increment above 17 °C.

Non-chilled larvae

Log_{10} of surviving L3 was highly significantly correlated with time for all treatments ($p < 0.001$). Survival parameters can be found in table 4.4. At 11, 13, 15 and 17°C, death rates were higher than in chilled larvae kept at the same temperatures. Also, between the non-chilled treatments, with each 2°C increase survival rates decreased significantly. No differences in mortality rate were found between constant 17°C and fluctuating 14-20°C treatments.

Temp. (°C)	-5	6 + frost	6	11	13	15	17	14-20	20	25	30
L_{50}	220 (5)	220 (5)	185 (5)	190 (5)	165 (5)	165 (5)	140 (5)	65 (5)	45 (5)	15 (5)	5 (5)
μ	0.00088 (0.00041)	0.00081 (0.00026)	0.0016 (0.00020)	0.0015 (0.00021)	0.0019 (0.00034)	0.0020 (0.00044)	0.0029 (0.00042)	0.0084 (0.0011)	0.011 (0.0010)	0.025 (0.0034)	0.137 (0.048)
P_{100}	0.84 (0.03)	0.73 (0.03)	0.76 (0.01)	0.77 (0.01)	0.73 (0.02)	0.72 (0.03)	0.57 (0.03)	0.21 (0.08)	0.12 (0.07)	0 (0.01)	0 (0.02)

Table 4.3 Survival of chilled larvae. L_{50} = time (in days) to 50% survival (rounded up in 5 day intervals); μ = the slope of the log linear regression line and equals the instantaneous daily mortality rate; P_{100} = the predicted proportion of larvae surviving for 100 days at a given temperature; 95% confidence intervals (plus or minus) are given between brackets.

Temp. (°C)	11	13	15	17	14-20
L_{50}	280 (5)	75 (5)	75 (5)	55 (5)	55 (5)
μ	0.00090 (0.00018)	0.0067 (0.00079)	0.0085 (0.00072)	0.010 (0.0010)	0.010 (0.0010)
P_{100}	0.85 (0.02)	0.36 (0.05)	0.29 (0.05)	0.13 (0.07)	0.15 (0.07)

Table 4.4 Survival of non-chilled larvae. L_{50} = time (in days) to 50% survival (rounded up in 5 day intervals); μ = the slope of the log linear regression line and equals the instantaneous daily mortality rate; P_{100} = the predicted proportion of a larva surviving for 100 days at a given temperature; 95% confidence intervals (plus or minus) are given between brackets.

4.3.5 Egg development and hatching at pasture

From 30-09-2005 to 3-11-2005 the minimum and maximum temperature both remained between 10 and 17°C, for a total of 35 days. In the following spring similar temperatures were experienced for 13 days, between 15-04-2006 and 27-04-2006. As figure 4.5 shows, both these periods were followed by peaks of larval emergence, both at the plots where eggs had been placed in the embryonated development stage and at plots where un-embryonated eggs in dung had been placed out. The model for daily development described above predicted that the latter eggs should have completed larvation by 12-08-2005. However, in comparison to the plots seeded with embryonated eggs, fewer larvae than expected were recovered, even if the percentages of non-developing eggs found in the laboratory experiments were deducted. The weeks after the dung was placed at pasture were very dry and the desiccated heaps disintegrated very slowly. On 10-10-2005 samples of dung remaining at pasture were examined and only perished and non-developing eggs were detected. It appears from fig. 4.5 that in autumn, either hatching took place over a longer period of time or larvae were longer lived than in spring.

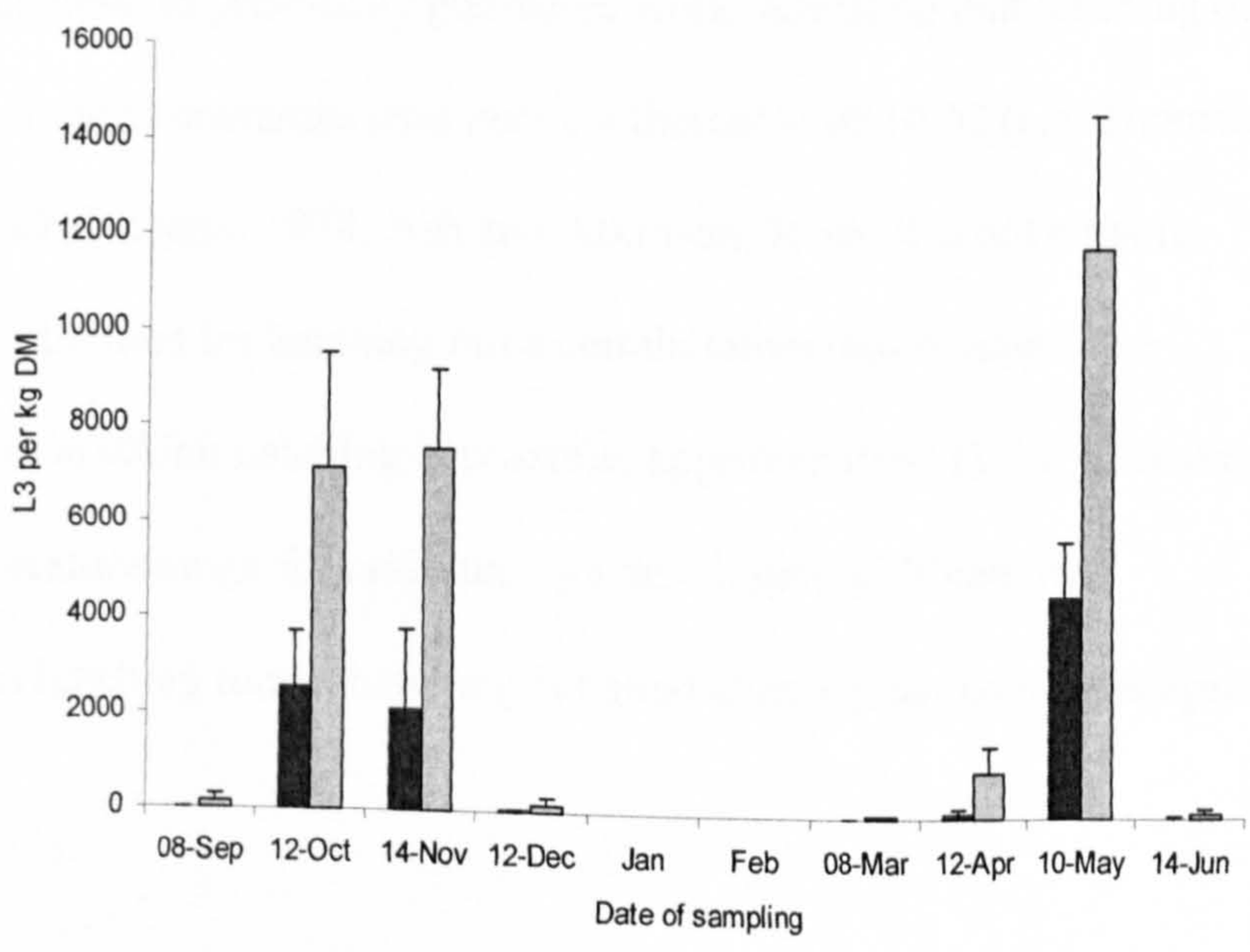


Fig. 4.5 The hatching of non-embryonated eggs put out at pasture on 07-07-2005 (black bars) as measured between 08-09-2005 and 14-06-2006, and of embryonated eggs put out 29-09-2005 (grey bars) measured between 12-10-2005 and 14-06-2005. On the vertical axis the average number of larvae recovered, expressed as L3 per kg dry matter of herbage, is given. The error bars represent the standard deviations (n=4).

4.4 Discussion

This chapter describes the vital rates and thresholds of the free-living stages of the parasite *Nematodirus battus* for the first time. In addition, other characteristics of epidemiological importance came to light during the laboratory and field experiments:

(1) Although consistently higher proportions of chilled than non-chilled eggs hatched, a substantial proportion of larvae was able to hatch without any prior chilling treatment. At a hatching temperature of 13°C, for example, 81% of the embryonated egg population hatched regardless of chilling experience while the remainder hatched only after chilling processes had been completed. This hatching of proportions of the population as non-chilled larvae in the laboratory, which was subsequently demonstrated at pasture, was also observed first by Gibson and Everett (1981) and later by Rickard *et al.* (1987, 1989).

(2) There is not only a lower temperature threshold for hatching but also an upper threshold. To my knowledge this is the first evidence for the existence of such a threshold in trichostrongyloids. Also, in contrast to previously published work indicating that hatching of chilled eggs only occurs when the temperature rises above a threshold of 10 °C (e.g. Thomas and Stevens, 1960; Christie, 1962; Dunn, 1978; Ash and Atkinson, 1986) it is not a rising temperature *per se* that is the stimulus for hatching but a certain temperature range. The range of constant temperatures at which hatching is possible, approximating 11-17°C, is very narrow and close to the temperature range for optimum egg development. When the temperature moves out of this hatching range, hatching is halted abruptly but continues again

as soon as hatching range temperatures are restored. Hatching appears to be a function of cumulative within-threshold temperature experience.

(3) When chilled egg populations experience temperatures within the hatching range, fewer eggs hatch towards the higher end of the hatching range. For those non-chilled eggs that hatch, this decrease is much more pronounced. Also, the timing of the hatch varies with the hatching temperature. The optimum temperature, maximizing both hatch magnitudes and hatching rates, is 13°C. The peculiar hatching behaviour of the parasite seems targeted towards maximizing the survival of hatched larvae. Survival times of chilled larvae were shorter above the hatching threshold. Death rates of non-chilled larvae were significantly higher than those of chilled larvae kept at the same temperatures, while they also increased with each 2°C increment within the hatching range. The difference in longevity of chilled and non-chilled larvae may be explained by the quality of their respective energy reserves. In parallel with many species of insect (Morrissey and Baust, 1976), *N. battus* larvae transform their lipid reserves into carbohydrates (trehalose) during the chilling process (Ash and Atkinson, 1983; Ash and Atkinson, 1986). Higher carbohydrate content has been linked with increased nematode longevity (Patel *et al.*1997; Patel and Wright, 1997).

(4) Regarding the influence of fluctuating day and night temperatures on the hatching behaviour of *N. battus*, hatching did not take place when temperatures fluctuated between temperatures below and within the hatching range while fluctuations within the range did not alter the expected hatching patterns. However, given the results of the hatching experiments at continuous temperatures, hatching in response to fluctuations around the upper threshold

gave unexpected results, significant hatching taking place. One explanation could be that, at fluctuating temperatures, hatching continues as long as the average temperature lies within the hatching range. It is also possible that hatching is induced by certain fluctuations around the upper threshold *per se*, for example as the result of increased activity of larvae as the result of these fluctuations. Hyperactivity of larvae in the eggs has been linked with hatching (Parkin, 1972) and in our experiments hatched larvae, at fluctuating 14-20°C, were observed to be more active than in any of the other treatments, while the larvae emerging from chilled eggs were significantly shorter lived than those kept at the mean of 17°C. Hatching as the result of these fluctuations could explain the presence of small numbers of larvae at pasture throughout the grazing season, as recorded by several workers (e.g. Graham *et al.* 1984; Thomas, 1991). However, in the incubator the fluctuations, taking place over 20 minutes, are unnaturally abrupt and the existence and importance of this type of hatching would have to be verified at pasture.

(5) None of the temperature-dependent death rates can explain the typical recovery rates of L3 from herbage, which are reduced to approximately 20% of the peak recovery within the first month following the peak and then gradually to zero over the following two months (Thomas, 1959b; Gibson, 1963; Boag and Thomas, 1975; Graham *et al.* 1984). *N. battus* L3 have been shown to be extremely resistant to desiccation (Parkin,1972) and therefore factors other than temperature and rainfall must contribute to larval death in temperate regions.

(6) From the magnitude and the pattern of the hatch of non-chilled eggs at 11°C, and survival rates of non-chilled larvae at 11°C, which were the only ones not to differ from the

rates of their chilled counterparts, it appears that 11°C is the upper threshold temperature for 'chilling' experience. In a minimum of four weeks the biochemical processes taking place during chilling can be completed.

(7) Apart from the hatching temperature, the hatching 'motivation' of the eggs is also influenced by the time that embryonated eggs spend in conditions adverse to hatching. For example, although the minimum time in which the chilling processes can be completed is 4 weeks, prolonged chilling can further increase the percentage of the egg population hatching. Extended spells at 20°C, however, decreased the percentage hatching, even though the eggs were shown to be viable as they hatched after chilling.

(8) Not only hatching but also egg development took place in a highly synchronized manner. At lower, and at fluctuating, temperatures, all eggs completed their development approximately within the same week. Development time of egg populations is therefore very well characterised by D_{50}.

(9) Survival rates of both embryonated and non-embryonated eggs, during long spells of continuous high temperatures, were much higher than those of hatched L3 larvae. However, although this shows that the parasite population will be able to survive high temperatures at pasture, it thrives at lower temperatures. This was demonstrated by the fact that, at temperatures in the lower part of the development range, higher proportions of eggs developed in a more synchronized manner.

(10) The standard techniques in use for determining the minimum development threshold (T_0) predicted development to cease at the highly unlikely value of -3°C. It is widely recognized that such linear extrapolation techniques do not necessarily represent the true T_0 (Ames and Turner, 2003). However, the predicted T_0 normally over-estimates the true figure but under-estimated it by 11.5°C for *N. battus*. Although predicted T_0 has proven to be reliable for certain sub-arctic parasites (Jenkins *et al.* 2006a), extrapolated T_0 values should always be verified in the laboratory.

(11) In line with the work of Salih and Grainger (1982) and Saunders *et al.* (2002) temperature fluctuations were shown to significantly increase development rates. At fluctuations quite accurately reflecting UK day and night soil temperatures during the spring and early summer, development was completed in approximately two-thirds of the expected time. These fluctuations did not affect the synchronization of egg development.

(12) At pasture, development and hatching appeared to take place in line with the findings of the laboratory experiments. *N. battus* eggs cannot develop in dung. This also emerged from work by Gibson and Everett (1981). Faecal breakdown rates, which are strongly influenced by local weather, may therefore play an important role in the epidemiology of this parasite.

The epidemiology of *Nematodirus battus* on farms in temperate regions can be better understood by taking into account these results. As was also shown by Thomas and Stevens (1960) and Gibson and Everett (1981), eggs resulting from spring infections will most likely be fully developed by the end of the summer. A proportion of these will hatch at the first

opportunity, in autumn, when minimum and maximum temperatures are within the hatching range for prolonged periods of time. The remaining eggs undergo chilling at pasture during the winter and hatch the following spring. As the minimum temperature for development lies within the hatching range, and temperatures fall in autumn, eggs deposited at pasture as the result of autumn infections are highly unlikely to be fully developed by the time the next window of opportunity for hatching arises in spring. Some of the autumn-deposited eggs will therefore hatch the following autumn, having not experienced chilling at the embryonated stage, and the remainder a year and a half after deposition, in the following spring. Thus, in general, a spring peak arises from eggs deposited the previous spring and the autumn before that, while an autumn peak results from eggs deposited during the spring of the same year and the autumn of the previous year. However, as it takes approximately 8 days for the first larvae to emerge and another 8-10 days for the hatching process to be completed, in some years, the temperature may rise above the upper hatching threshold or fall below the lower threshold before all eggs complete hatching. In this case a proportion of the eggs may carry over to the next window of opportunity. This may be the next spring or autumn but could also be a cold spell during the summer. Although the effects of long-term survival of eggs at pasture on the ability of larvae to infect the host have not been established, and larval emergence does not have to equal transmission (Rickard *et al.* 1989), the above appears to explain the large between-year variations of not only the magnitude of spring and autumn peaks but also of the number of reported cases of nematodirosis (chapter 2). It also suggests that viewing nematode population dynamics in varying environments in terms of limited windows of opportunity for transmission, rather than more gradual flows through development stages driven by undulating mean temperatures, might be helpful in

characterizing and predicting epidemiological patterns (Morgan *et al.* 2004).

The minimum egg development threshold value of 11.5°C is approximately twice as high as that of *Teladorsagia circumcincta* (Young *et al.* 1980), also an arctic-adapted Trichostrongyloid, and perhaps even higher than that of *Haemonchus contortus* (Gibson and Everett, 1976a; Besier and Dunsmore, 1993), a species of tropical origin. The patterns of within-threshold egg development rates, the hatching thresholds and rates themselves, and the larval survival characteristics also do not seem to fit a parasite experiencing summers with maximum temperatures of 20-30°C and winters with minimum temperatures just below zero. The supercooling point of embryonated eggs before chilling has been shown to be as low as minus 34°C (Ash and Atkinson, 1986) and thus the mechanism of lowering the supercooling point further by accumulating trehalose during chilling seems irrelevant to over winter survival in the temperate regions. However, the minimum development temperature fits that of other parasites of sub-arctic origin needing similar numbers of degree-days for the completion of their non-parasitic development phase (Schjetlein and Skorping, 1995; Kutz *et al.* 2001). Schjetlein and Skorping (1995) showed that there is likely to be adaptation of sub-arctic parasites towards these higher minimum temperature thresholds as they may prevent nematodes from entering the winter at developmental stages most sensitive to very low temperatures. Following this hypothesis, a fall below 11.5°C would mark the end of the arctic summer and the point at which development would certainly not be completed before the start of the period of permanent frost. During the arctic autumn, soil temperatures remain between 10 and 0°C for approximately one month, before dipping far below zero, according to our findings leaving enough time to complete the processes leading to increased cold

hardiness (Ash and Atkinson, 1986) of embryonated eggs. For most of the arctic summer, during which day-night temperature fluctuations are very small, temperatures normally stay within the 10-15°C range and rise above 20°C only during exceptionally warm summers (Kutz *et al.* 2002). At these temperatures all the responses to temperature described in this paper fall into place. This work therefore supports the hypothesis that *N. battus* has an Arctic origin. In the temperate regions, the high temperature threshold for development, the inability to apply thermal energy at higher temperatures, and the narrow hatching range are unlikely to be beneficial to the parasite. Thirty-five years ago, Parkin (1972) also described that at 20 and 25°C "the majority of the eggs did not reach the third stage". It would appear that the adaptation of nematode enzyme temperature thresholds to new environments either falls beyond the scope of genetic plasticity or are slow to occur. The detailed studies by Troell *et al.* (2003, 2005, 2006), addressing possible adaptations of *Haemonchus contortus* to colder environments, found evidence for such adaptations neither at phenotypic nor at genotypic level. These findings may have important implications for the adaptability of parasites to future climate change.

Rose and Jacobs (1990), working in sub-arctic Greenland, showed that the epidemiology of *Nematodirus spathiger*, *N. abnormalis* and *N. helvetianus* in these regions is characterized by low numbers of larvae spread out over large areas, leading only to trickle-type infections. In such environments with low host densities larval survival is likely to be crucial to the persistence of parasite populations and there is unlikely to be selection for the hatching of non-chilled eggs, whose larvae survive less well than those hatching from chilled eggs. The synchronized hatch of larvae has, in the temperate regions, been interpreted as a

synchronization of maximum larval availability with the presence of susceptible hosts, i.e. a strategy targeted at mass invasion of the host. However, it may originally have been targeted at maximizing the probability of being ingested at all, by ensuring that larvae were present as soon as the herbage started to grow, and throughout the grazing season. The mass invasion of hosts, sometimes leading to their death, may just be an artefact of this behaviour in situations of unnaturally high host density. As host densities are determinants of parasite abundance (Arneberg *et al.* 1998), very low-level abundance in free roaming non-domesticated animals could explain why the origins of *Nematodirus battus* have remained enigmatic (Jansen, 1973). Increased host density, in combination with increased predictability of host grazing behaviour, and different climatic conditions impacting on larval survival, are likely to have made a hatching strategy that maximizes larval survival redundant. Instead, the common practice on UK farms of avoiding grazing of young lambs on pasture used by lambs the previous spring, in an attempt to evade infection with *N. battus* larvae emerging from the previous year's egg deposition (Black, 1959), may have made transmission at other times of the year a necessity for the persistence of the species. Increased between-year fluctuation in spring hatching conditions in temperate areas compared with the Arctic would at the same time cause greater uncertainty in hatching conditions. If all eggs required chilling before hatching, and spring temperature rose above the upper threshold before hatching was completed, larval emergence would be delayed by at least a full year. By ensuring that a proportion of eggs hatch without chilling, larvae will be available to infect hosts in autumn and secure one worm generation per year, improving the chances of persistence and population growth. Bet-hedging strategies have been linked with increased environmental uncertainty in many systems (e.g. Philippi and Seger, 1989;

Fenton and Hudson, 2002; Meyers and Bull, 2002), and phenotypic plasticity in *N. battus* hatching requirements may be an example of such a strategy. Mass invasion of young hosts by larvae in the spring peak leads either to rapid worm expulsion within three weeks of the initial infection in the majority of hosts (Gibson and Everett, 1963; Taylor and Thomas, 1986) or to host death before eggs are produced. Lower level, trickle-type, infections have been shown to enhance total egg production in other gastrointestinal nematodes (Grenfell *et al.* 1987b, Barger and Le Jambre, 1988, Christensen *et al.* 1997), and these eggs mitigate the risk of extinction of *N. battus* populations that otherwise rely on a single mass infection event each year. There are, on the other hand, clear advantages to a mass hatch. By infecting hosts in spring before other gastrointestinal parasite species, *N. battus* could avoid competition and cross-immunity. On many farms where *N. battus* was introduced *N. spathiger* and especially *N. filicollis* were already established and successful but quickly gave way to *N. battus*, which became the dominating species (Helle, 1969). Mass invasion at sub-lethal levels could also promote maturation of a large number of worms and production of eggs before the time-lagged host immune response becomes effective. Retention of mass hatching, alongside the release of a smaller proportion of larvae outside the main transmission window, might represent the best chance of parasite persistence in an unpredictable environment.

If *N. battus* is indeed bet-hedging, differences in selective pressure between regions in the UK, or even differences in the management of neighbouring farms, might result in differences in hatching behaviour between field isolates. Most of the earlier work on the parasite did not describe significant hatching without chilling or larval appearance on pasture

in autumn (Thomas, 1959a; Thomas and Stevens, 1960; Gibson, 1963; Boag and Thomas, 1975). Other workers (Parkin, 1972; Ash and Atkinson, 1986) detected a significant hatch in the non-chilled treatments but did not comment on this. Gibson and Everett (1981) described significant autumn hatching at pasture for the first time. A few years after this publication, increased larval availability in autumn leading to deaths in lambs was signaled in the veterinary press (McKellar *et al.* 1983; Rodger, 1983). Thomas (1991) researched the hatching patterns of two isolates, one lowland and one hillside, on both lowland and upland farms, and remarked on the distinct differences in hatching patterns. Thomas proposed that differences in hatching patterns may be heritable and plastic.

From a more global perspective, bet-hedging has two distinct advantages for *N. battus*. Firstly, the ability to hatch without chilling means that the parasite is not dependent for its survival on climatic regions where the temperature drops to or below 11°C for at least one month each winter. This potentially enables a massive expansion of global habitats. *N. battus*, whose eggs are also able to survive temperatures as low as minus 50 °C (Ash and Atkinson, 1986), could in principle become established and persist in any region where temperatures stay below 25°C for at least 6-7 weeks per year and hatching temperatures stay within the 11-17 °C range for 2-3 weeks per year. Prolonged periods of temperatures of 25-30 °C pose little threat to the eggs. High temperatures will increase death rates of infective larvae but at high host densities this is not necessarily followed by extinction of the parasite population. The establishment of *N. battus* on Sicily (Torina *et al.* 2004) and Mexico (Sanchez and Quiroz Romero, 1993) most likely demonstrates expansion outside the area in which chilling of eggs takes place. Adaptation to the temperate regions may therefore have

been a stepping stone to further southward expansion. Secondly, in the face of climate change this parasite, originally thriving in the cold, has a much higher chance of persistence in temperate areas as a result of its ability to hatch without chilling. Increases in maximum temperatures, and day-night fluctuations over a greater temperature range, will quite likely lead to a decreased number of days on which hatching is possible. In this new environment with increased hatching uncertainty the bet-hedging parasite will be more likely to complete its hatch and enhance prospects for host infection and persistence.

4.5 Conclusions

The development and hatching thresholds of *N. battus* described here would suit this parasite in the Arctic. In this environment, the thresholds, and the hatching of larvae after chilling only, would appear to be adapted to minimise temperature-driven larval death rates. In the United Kingdom, as the spring hatch of the parasite often coincided with the presence of young lambs, the parasite benefited greatly from the predictability of grazing patterns, and increased host-density, on commercial farms. However, in this new environment, as temperature rises occurred more quickly, the upper threshold may have become limiting for the completion of the hatching process. At the same time factors other than temperature became limiting for larval survival, removing the benefit of the hatching of chilled larvae with longer lasting energy reserves. The proportion of eggs hatching with or without chilling appears to be a trait more plastic than the upper threshold for hatching. Therefore, the parasite has had to utilize other transmission windows instead. As, in the autumn, hosts with

a higher level of immuno-competence are present at pasture, the increased probability of hatching was most likely traded off with a decreased offspring per worm. A positive side-effect of the adaptations to the climate of the temperate regions may have been an expansion of the global range, further south.

Although the epidemiology of this parasite is dramatically different from those modelled in the previous chapter, there are great similarities between the species in the temperate regions. Temperature thresholds appear to be the key to the epidemiological patterns observed at pasture. They are likely to be the main constraint for increasing the success of parasites, yet they appear not easily adaptable. Climate change, in its widest sense, increases the importance of the autumn as part of the window of parasite transmission. Therefore, apart from absolute changes in the number of parasites at pasture, climate change is likely to alter host-parasite interaction. These alterations include higher challenges to older animals.

Chapter 5- Variation in hatching behaviour of *Nematodirus* populations

5.1 Introduction

Phenotypic traits often differ between conspecific populations inhabiting different environments (Jarrett, 2008). These differences may reflect evolution of genotypes, for example towards increasing survival in a certain environment, or different expressions of the same genotype triggered by environmental conditions (Jarrett, 2008). The concept of phenotypic plasticity is widely accepted in parasitology (Stearns, 1992; Poulin, 2007). The capability of a given genotype to produce a variety of phenotypes allows for fast responses to environmental changes (Kirkpatrick and Lande, 1989). If these changes persist in time, a shift in genotypic frequencies may subsequently follow and therefore the two processes can be difficult to distinguish (Poulin, 2007).

Evidence for conspecific variability in phenotypic traits, and phenotypic plasticity, has largely emerged from studies reporting expressions of life history traits of one isolate in one (Shostak and Dick, 1987; Szalai and Dick, 1989; Stear *et al.* 1997; Fenton *et al.* 2006) or several (Vignoles *et al.* 2004) host species. Studies comparing life history traits across several field isolates of the same species, or sister species, are very rare (Almeida *et al.* 2007). This chapter explores evidence for conspecific and interspecific variability in *Nematodirus* life history traits, with particular focus on hatching behaviour, and fecundity. The species *N. filicollis* is also included in the study to investigate constraints on the persistence of *Nematodirus* species in new environments.

5.1.1 Fecundity

"In many parasites, egg volume may reflect not only the amount of energy invested in each offspring, but also the amount of resources available to each offspring for host finding. Intraspecific variation in egg volume may thus mirror variability in the infection strategies of parasites." (Poulin, 2007). The trait egg size may be under selective pressure from the environment. In parasitic insects, a very high degree of egg size variability has been related to the diversity of host plants in the parasite's surroundings (Mizumoto and Nakasuji, 2007). Poulin and Hamilton (2000) showed that variability in egg sizes of trematodes may be positively correlated with increased spatial heterogeneity of the external environment. Nematodirae are low-fecund parasites, producing relatively large eggs which protect the larvae from environmental noxes. In the previous chapter it was made likely that, in the Arctic environment, the hatching of eggs of these parasites was targeted at maximising larval survival potential. As the larvae, unlike most other parasitic nematodes, are not able to eat during the developmental stages all energy present in the L3-stage larva must already be present in the egg. Therefore it is likely that this strategy represents a high energy input per offspring, and a limitation on fecundity. In the temperate regions, hatched larvae die rapidly at pasture and large carbohydrate reserves do not appear to be advantageous. As the probability of transmission of offspring of higher fecund, smaller egg producing, worms would be just as great as that of other worms their relative importance in the population would be expected to increase. It may therefore be hypothesized that environmental changes nullifying benefits of extra energy reserves lead to increased fecundity. Such increases in fecundity could, in turn, partially make up for lower establishment rates of autumn-hatched

larvae in immune animals (Israf *et al.* 1996). Quantifying existing heterogeneity in the volume of *Nematodirus* egg populations is a first step in testing this hypothesis.

5.1.2 N. battus hatching behaviour

Heterogeneity in parasite populations has been associated with environmental, including climatic, uncertainty (Fenton and Hudson, 2002; Meyers and Bull, 2002). In light of the upper threshold for hatching, the probability of *N. battus* being able to complete its hatching process may vary in different climatic localities. In chapter 4 it was proposed that the proportion of *N. battus* larvae hatching without chilling may be influenced by this probability. This could in part explain the regional differences in epidemiology described in chapter 2. Thus, in general, in Scotland a higher proportion of eggs would be expected to hatch after chilling only, i.e. in spring. However, host availability also represents uncertainty and is therefore a co-determinant of the evolution of parasite life histories (Crossan *et al.* 2007). On UK farms, heterogeneity of worm populations may therefore also be a function of the predictability of grazing patterns and the number of pasture plots available for grazing. If climatic differences and/or differences in farm management drive the hatching behaviour of *N. battus* significant differences in the proportions of larvae hatching without chilling may occur between regions or even between neighbouring farms. As the degree to which non-chilled eggs hatch is likely to have an important impact on *N. battus* epidemiology, for mathematical models of the ecology of the parasite, it is important to know whether differences indeed occur and, if so, at which spatial scale.

5.1.3 Inter-specific differences in transmission success

If the hatching of *N. battus* can indeed be fine-tuned to local on-farm circumstances the question arises whether this, in the UK, is likely to account for the relative success of the species over the sister species *Nematodirus filicollis*. Indeed, given the recent history of the two parasites, it would seem appropriate to investigate what actually constitutes 'success' within the genus *Nematodirus* Ransom, 1907: in the UK, *N. filicollis* and *N. spathiger* were the dominant species (Spedding *et al.* 1958) but, after its introduction, *N. battus* rapidly took over this role across the country. This also happened after the introduction of the species to the Netherlands in 1978 (Eysker, M., personal communication). Helle (1969) described how *N. battus* became the dominating species within two to three years after its arrival on a sheep farm in Norway, seemingly suppressing the numbers of the other two *Nematodirus* species present on the farm. However, there is a paradox between the success of *N. battus* in a few countries within the temperate regions of the Northern Hemisphere on the one hand and the global spread of many other *Nematodirus* parasites on the other. After the initial distribution of the genus, starting in Beringia (Hoberg, 2005), species like *N. helvetianus* and *N. spathiger* have proved to be highly successful parasites in extreme environments all over the world (Marquardt *et al.* 1959). It is thought the eggs of these two species hatch whenever their development to the L3 stage is completed (Herlich, 1954; Kates and Turner, 1955), resembling general trichostrongyloid ecology only with a longer development phase. *N. filicollis* has been shown to have similar hatching characteristics to *N. battus* in terms of a delayed hatch after the completion of egg development (Thomas, 1959a; Thomas, 1959b; Christie, 1962; Gibson, 1963). The role chilling experience plays in the hatching of *N. filicollis* eggs has not been researched explicitly in the laboratory but field

Country		Hosts	Reference
Iceland		sheep	Richter, 2002
		goats	Kristmundsson, 2000
Poland		sheep	Gruner *et al.* 1998
Germany		sheep	Rehbein *et al.* 1998
Switzerland		camelids	Hertzberg & Kohler, 2006
Hungary		mouflon	Takacs, 2003
		fallow deer	Takacs, 2000
Bulgaria		roe deer	Svilenov *et al.* 1985
France	- Mediterranean	sheep	Mounport *et al.* 1990
Spain	- Central	goats	Valcarcel *et al.* 1999
	- Southeast	gazelles	Ortiz *et al.* 2001
Italy	- Central	mouflon	Magi *et al.* 2002
		chamois*	Genchi *et al.* 1982
	- Sicily	sheep/goats	Torina *et al.* 2004
Iraq		cattle	Al-Dulaimi *et al.* 1986
USA		sheep	Rickard *et al.* 1987
Chile	- South	alpacas	Valenzuela *et al.* 1998
New Zealand		chamois*, goats, sheep, cattle, tahr**	McKenna, 1997

Table 5.1 Geographical distribution and host species of *Nematodirus filicollis*. This list contains most published isolations of the species but it not extensive. * *Rupicapra rupicapra*, ** *Hemitragus jemlahicus*

studies (e.g. Boag and Thomas, 1975; Gibson and Everett, 1976b) strongly suggest that large parts of egg populations hatch without chilling. In contrast to *N. battus*, *N. filicollis* has not only established itself extensively in the temperate regions of the Northern Hemisphere, but also its sub-tropical parts and beyond, all over the world (see table 5.1). It is unlikely that this can be attributed to a wider host range. While sheep are regarded as the main host, *N. battus* has, like *N.filicollis* (see table 5.1), been found in a wide range of hosts including llamas (Bishop and Rickard, 1987), and camels (El-Wahed and Mohammad, 2005) and thrives in young cattle (Coop *et al.* 1991). Moreover, *N. battus* and *N. spathiger* are the only species for which patent infections have been realised in rabbits while *N. battus* has appeared to be the species better adapted to this experimental, non-ruminant, host (Gallie, 1972; Audebert *et al.* 2004). It appears all widely distributed members of the genus Nematodirus Ransom,1907 express low host-specificity.

Therefore, the question arises whether *N. filicollis* is better adapted to (sub)tropical environments and, if so, what this adaptation constitutes. Neither vital temperature thresholds nor the exact hatching behaviour of *N. fillicollis* have been described. As not only temperature thresholds, but also variability in hatching behaviour, appear to determine *N. battus* epidemiology, what role do these factors play for this sister species? A study of the species may show possible ways in which *N. battus* can adapt to climate change.

The general aims of this chapter are therefore:

(i) to establish the degree to which non-chilled *Nematodirus* eggs hatch varies between field isolates of the same species and between species

(ii) to identify the key determinant(s), other than host availability, for successful *N.battus* transmission in the UK and in warmer environments

(iii) to test whether there is significant conspecific variability in egg volume.

5.2 Materials and methods

In February 2006 young lambs, born in December and grazing on the farm from which the *N. battus* isolate described in chapter 4 had been harvested, showed patent *N. filicollis* infections while no other *Nematodirus* eggs, and only up to 50 eggs per gram of other trichostrongyloids, were detected in their dung. Fresh dung was subsequently harvested at pasture.

During Spring 2006 contacts in Scotland sent eggs of three *N. battus* isolates (see table 5.2). Isolates 1 and 2 had been identified as *N. battus* on adult worm morphology, in at the Scottish Agricultural College (St. Boswells). Isolate 3 had been identified on egg morphology, and this was later confirmed on larval morphology (see below).

Assuming hatching behaviour may vary from farm to farm, when comparing the behaviour of discrete *N. battus* field populations, mixing of populations may give erratic hatching results. New *N. battus* field strains could arrive on a farm either when animals are bought in or when grazing is shared with neighbouring farms. The three farms had not bought in

replacement ewes for ten years, and did not share pasture with neighbouring farms.

Eggs from all 4 *Nematodirus* isolates were freed from dung and transferred to a watery suspension in the manner described in chapter 4.

Scottish isolate	Farm location	Upland/ lowland	Month of lambing	Recovery of eggs	Grazing management
1	Borders (St. Boswells)	lowland	March	post mortem (1 animal)	No
2	Borders (St. Boswells)	lowland	March	post mortem (1 animal)	No
3	North Borders (Edinburgh)	hillside	March	dung from pasture (lambs)	No

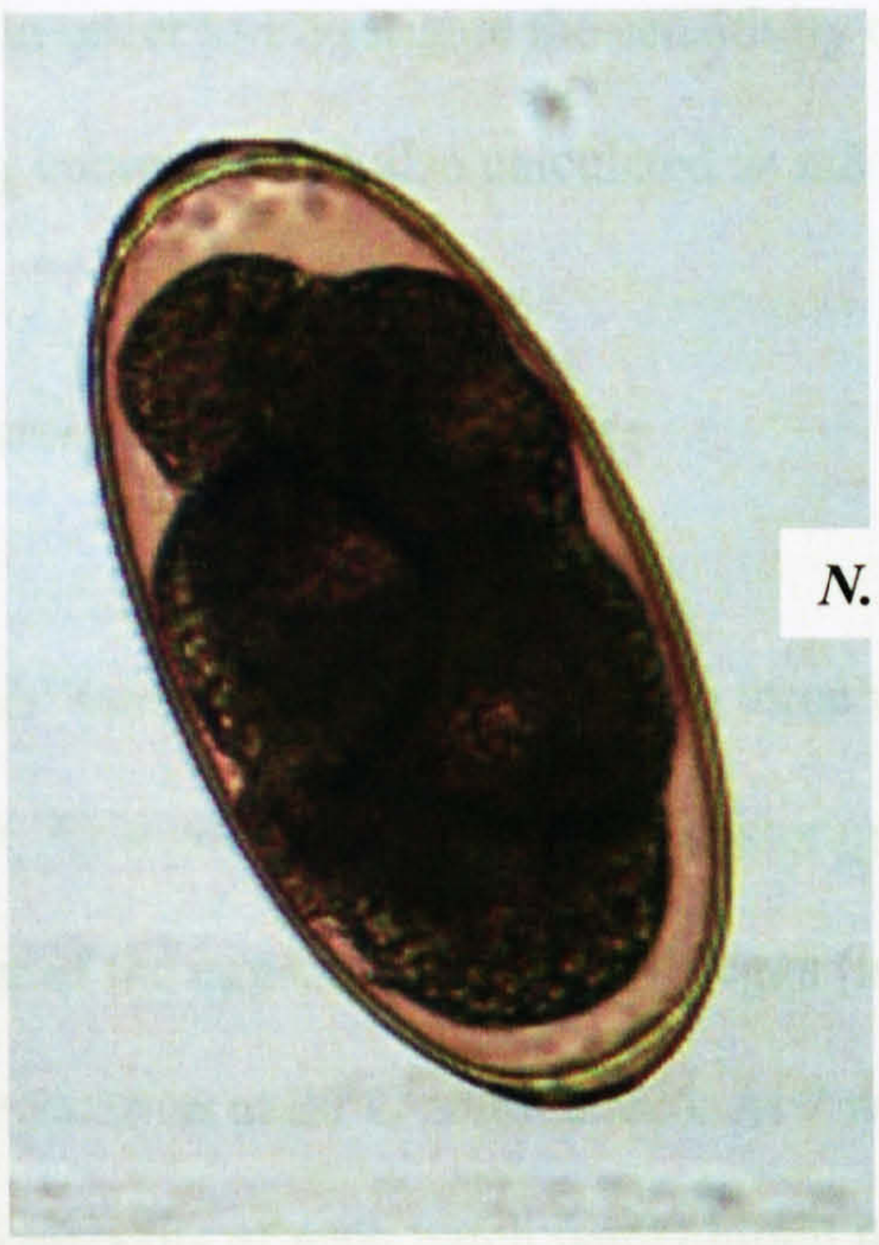
Table 5.2 Scottish field isolates. 'Grazing management' shows whether the general rule of, during nematodirosis risk months, not grazing lambs on pasture grazed by lambs around the same time in the previous year is strictly adhered to.

5.2.1 Purity of culture and egg measurements

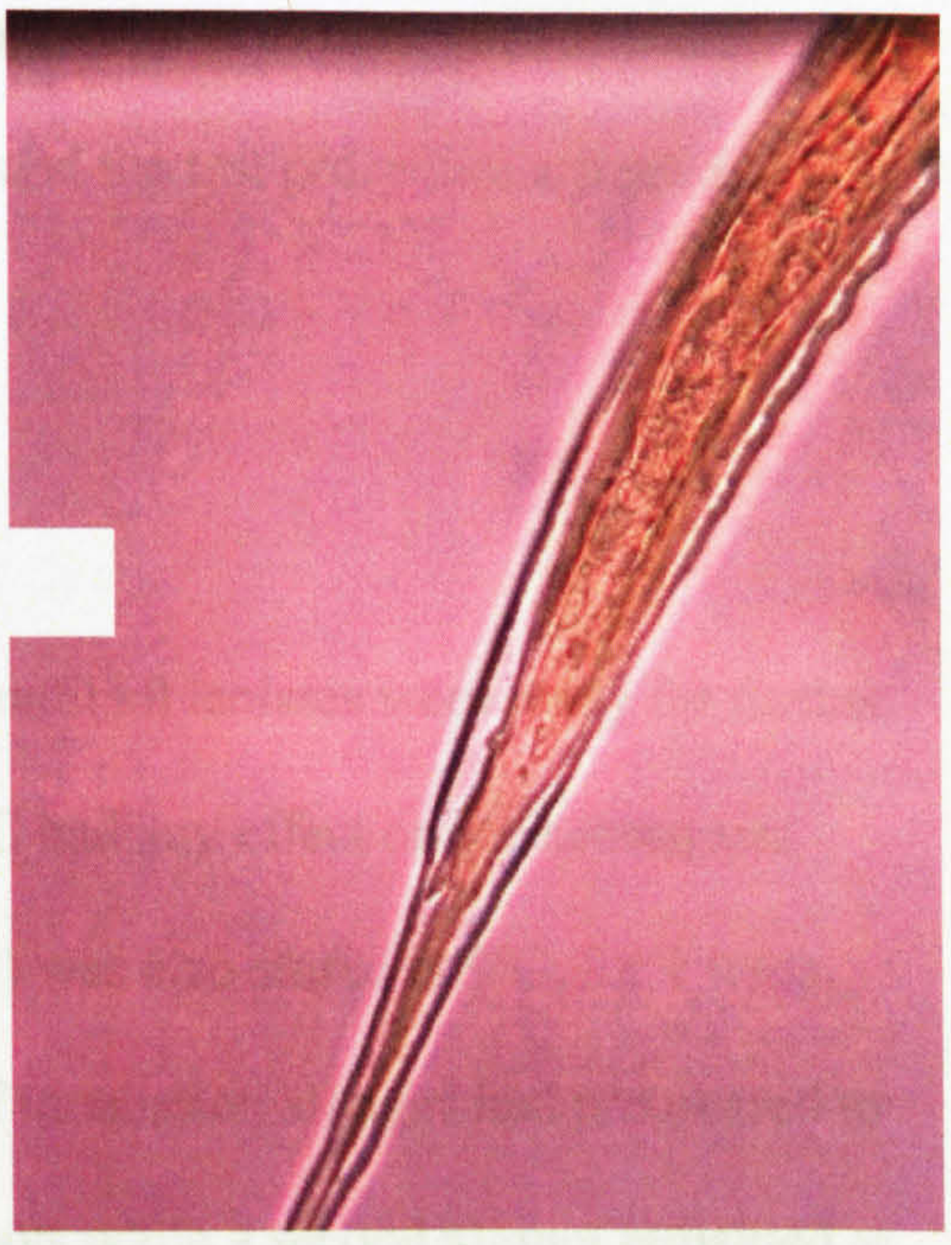
After a detailed inspection of thousands of eggs recovered from Scottish isolates it was concluded that the suspensions did not contain any *Nematodirus* eggs other than *Nematodirus battus*. However, amongst the *N. filicollis* eggs some *N. battus* eggs were identified. Therefore, 4 times 500 of the eggs in the *N. filicollis* suspension were counted and the number of *N. battus* eggs scored. *N. battus* eggs can easily be distinguished from *N.filicollis* eggs (Thomas, 1959a): 1. *N. battus* eggs have a brown egg shell while the

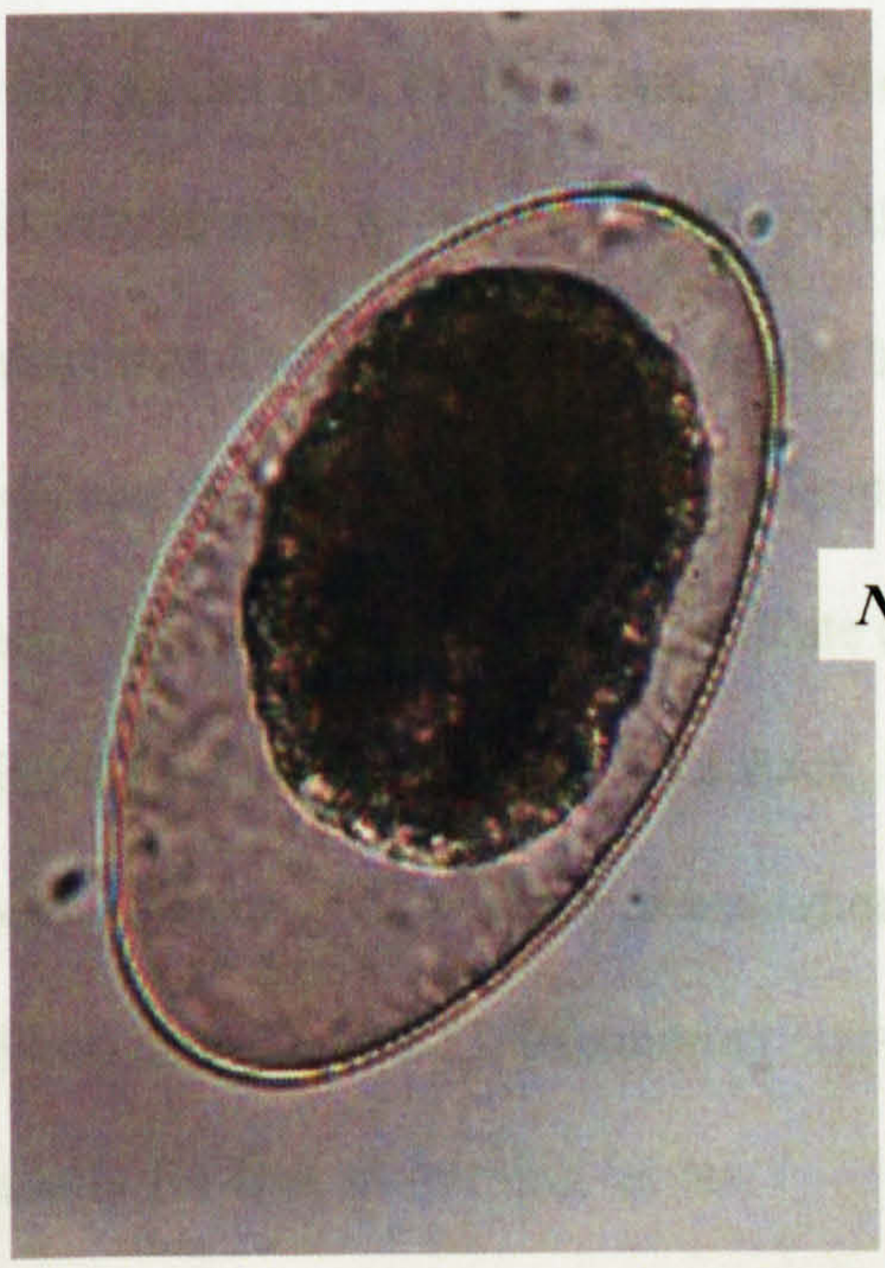
eggshell of *N. filicollis* is normally colourless: 2. *N. battus* eggs are longer and narrower; 3. A large part of the side ends of *N. battus* eggs, when viewed under a microscope, appears to be parallel to each other; 4.Assuming that the perivitelline membrane lies directly against the egg shell, the eggs shell of *N. battus* appears to be thicker, especially at the poles (see fig. 5.1). During the experiment, while studying the hatching of eggs, the purity of the field was confirmed on larval morphology. The infectious larvae of *N. battus* and *N. filicollis* isolates are readily distinguished on differences in size and the characteristic shapes of the tail (see fig 5.1), even under 10 x 10 magnification.

The maximum length (L) and width (W) of 100 eggs of each of the three Scottish isolates, the isolate described in chapter 4 (isolate 'Amos'), and the *N. filicollis* isolate, were measured with a digital, freshly calibrated, Motic® microscope and Motic Versatile Software®, version 3.0 (both by Micro-Optic Industrial Group Co. Ltd, UK). In order not to select for heavier eggs, clusters of eggs were carefully pipetted from the bottom of the Petri dishes holding them. For comparison, the measurements of 71 *N. filicollis* eggs published by Shorb, 1940 were extracted from the graph. Preston (1974) mathematically described the approximation of the volume of bird eggs. The shape of eggs can be approximated as somewhere in between that of a cylinder (volume = $\pi/4 * LW^2$) and that of a bicone (volume = $\pi/12 * LW^2$). On length (L) and width (W) measurements, their volume is best estimated as $\pi/6 * LW^2$. The estimated egg volume was thus computed for the four *N. battus* and the two *N. filicollis* isolates and all compared in a one-way ANOVA, with factor isolate, and Tukey's pairwise comparison. As the eggs of *N. filicollis* are more spherical in shape, using the same formula for the volume estimation of these eggs may lead to their relative over-



N. battus





N. filicollis

Figure 5.1 *Nematodirus* eggs and infectious larvae. The *N. battus* egg is brown, has more parallel sides (i.e. is less spherical) and a thicker egg shell. The tail of *N. battus* larvae extends into the tail sheath and has two characteristic notches; *N. filicollis* larvae have an empty tail sheath and a blunt, forked, tail end.

estimation. In order to investigate the sensitivity of the egg volume to these differences *N. filicollis* egg volumes were also calculated as $\pi/8 * LW^2$ and the test procedure repeated.

5.2.2 The hatching of N. battus isolates

Eggs in water were stored at 9°C, for up to three weeks, until all isolates were ready for further experiments. As it was not known whether storage of 9°C had any effect on the subsequent development of the eggs, the last batch of eggs (isolate 3) was also stored at 9°C, for 1 week, before embryonation at 20°C commenced. At 7 weeks of incubation all eggs had progressed to the L3 stage aliquots of each batch were chilled for 29 days while a second aliquot remained at 20°C for the same length of time. After these 29 days, eggs were pipetted into wells as described in chapter 4 and placed at 9, 11,13,15 and 17°C. In order to minimise the effects of small differences in temperature within one incubator, the three sets of plates were, daily, randomly spatially re-distributed over one shelf. Distances between plates of the different isolates were kept to a minimum by stacking individual replicates of each different isolate (making three stacks of three plates, each containing a replicate of three isolates). Replicates were removed from, and placed back in, an incubator in a stack. The plates were counted on day 3 and then every other day up to day 31. In order to test whether further hatching occurred all plates were counted again on days 48 and 63. Remaining eggs were put at 20°C and the Petri dishes were scanned regularly for free swimming larvae.

The arcsine-transformed proportions of eggs of the three isolates that had hatched on day 31 were, for the non-chilled treatments and for the chilled treatments, analysed in a two-way

ANOVA's with factors temperature and isolate. In order to assess whether chilling at 11°C, and subsequent further hatching, occurred the arcsine-transformed proportions of hatched eggs on days 31 and 63 were, for each isolate, compared in a t-test and a Bonferoni correction applied. Differences in time taken to the completion of 50% of the hatch were analysed in a multifactorial ANOVA with factors 'chill', 'temperature' and 'isolate'.

5.2.3 N. filicollis thresholds

A somewhat reduced set of the experiments described for *N. battus* in the previous chapter was carried out on the *N. filicollis* eggs and larvae. *N. filicollis* data were analysed in exactly the same manner.

Development

Fresh *N. filicollis* eggs were incubated at the continuous temperatures described in chapter 4, in the same incubators and at the same time as the *N. battus* eggs, and development followed in the same manner. Only one incubator, set at 12°C, was added to the experiment and development of *N.filicollis* was not followed at fluctuating temperatures.

Pre-hatch larval survival at 20°C

Three replicas of approximately 500 embryonated eggs were left at 20°C for 12 weeks, then transferred to a chilling temperature of 4°C for 10 weeks, pipetted into wells, and allowed to hatch at 15°C. At the hatching temperature of 15°C, the number of hatched larvae were counted on days 4,6, 8 and 10.

Hatching

Eggs were incubated at 20°C for six weeks, at which time all developing eggs had reached the L3 stage. The suspension was thoroughly mixed and split into two aliquots, one half remaining at 20°C and the other half chilled at a constant 4°C. After 33 days, eggs of both treatments were pipetted into wells. Three replicas of approximately 500 eggs were put at continuous hatching temperatures of 4, 6, 9, 11,13,15,17 and 20°C as well as 12-hourly fluctuating temperatures of 7 and 13, 11 and 15, and 14 and 20°C. During a heat wave, the 11-15°C incubator broke down and this treatment had to be abandoned. Larvae were counted daily for 8 days and then again on days 12 and 20.

Temperature threshold for chilling experience

Three replicas of non-chilled eggs were kept at 6, 8 and 11°C for 56 days, then transferred to 13°C, and the number of hatched larvae counted on day 8.

Survival

Larvae that had hatched at 13°C were divided into Petri dishes and placed at temperatures of 9, 11, 13, 15, 17, 20, 25 and 30°C, as well as 12-hourly 14-20°C fluctuation, and followed for 140 days as described in the previous chapter.

5.3 Results

5.3.1 Purity of culture and egg measurements

Scoring of batches of eggs revealed the *N.filicollis* culture to consist of 95% (range 93-97) *N.filicollis* eggs and 5% *N.battus* eggs.

The egg measurements and volumes are given in table 5.3. If the same formula for egg volume was applied to both species, egg volumes varied significantly between isolates ($F_{5,599} = 97.01$, $p < 0.001$). Egg volumes of Scottish isolates 1 and 3 could not be separated ($p = 0.057$) but both were smaller than those of Scottish isolate 2 and of the Amos isolate ($p \leq 0.022$). Scottish isolate 2 and the Amos isolate were statistically similar in size ($p = 0.748$). Volumes of both *filicollis* isolates were very similar to each other ($p = 0.996$) but significantly larger than those of all *battus* isolates ($p < 0.001$).

If the formula $\pi/8 * LW^2$ was applied to the *N.filicollis* isolates differences between isolates were still significant ($F_{5,599} = 18.36$, $p < 0.001$) but both *N.filicollis* isolates became statistically inseparable from *N. battus* isolates Scottish 2 and Amos ($p \geq 0.114$).

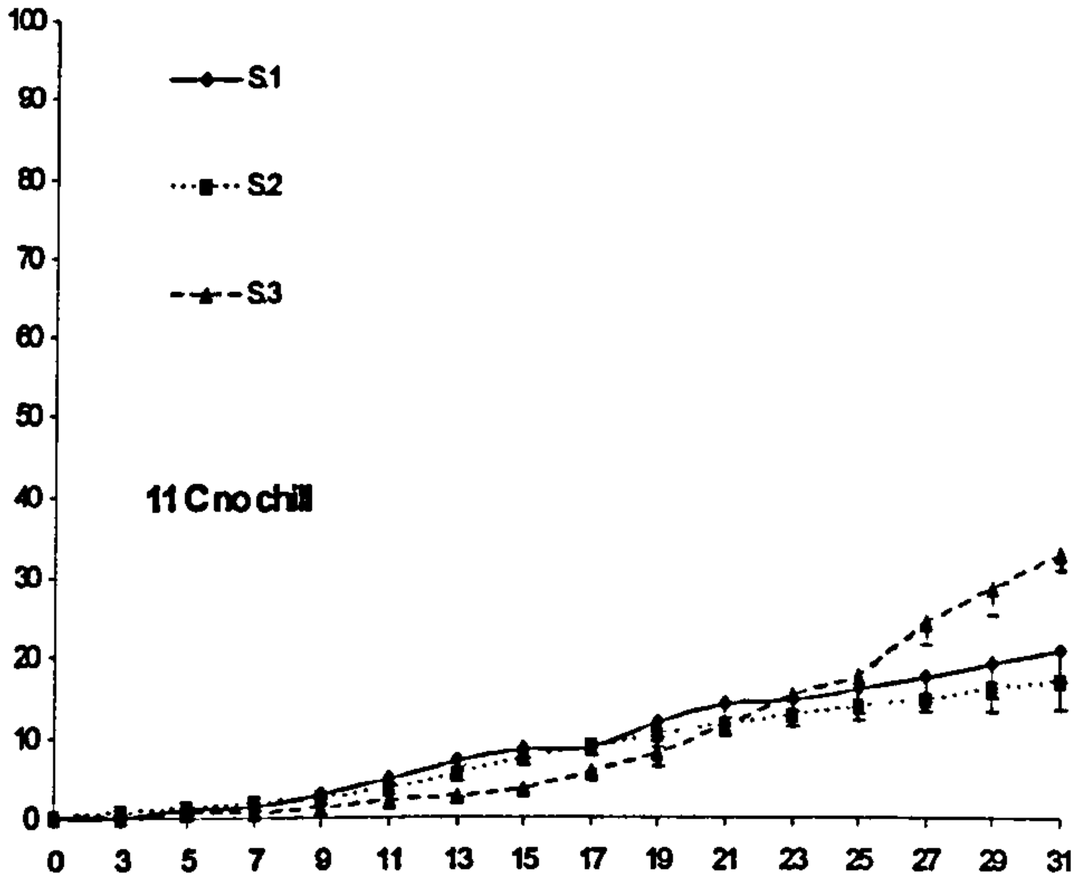
5.3.2 The hatching of N. battus isolates

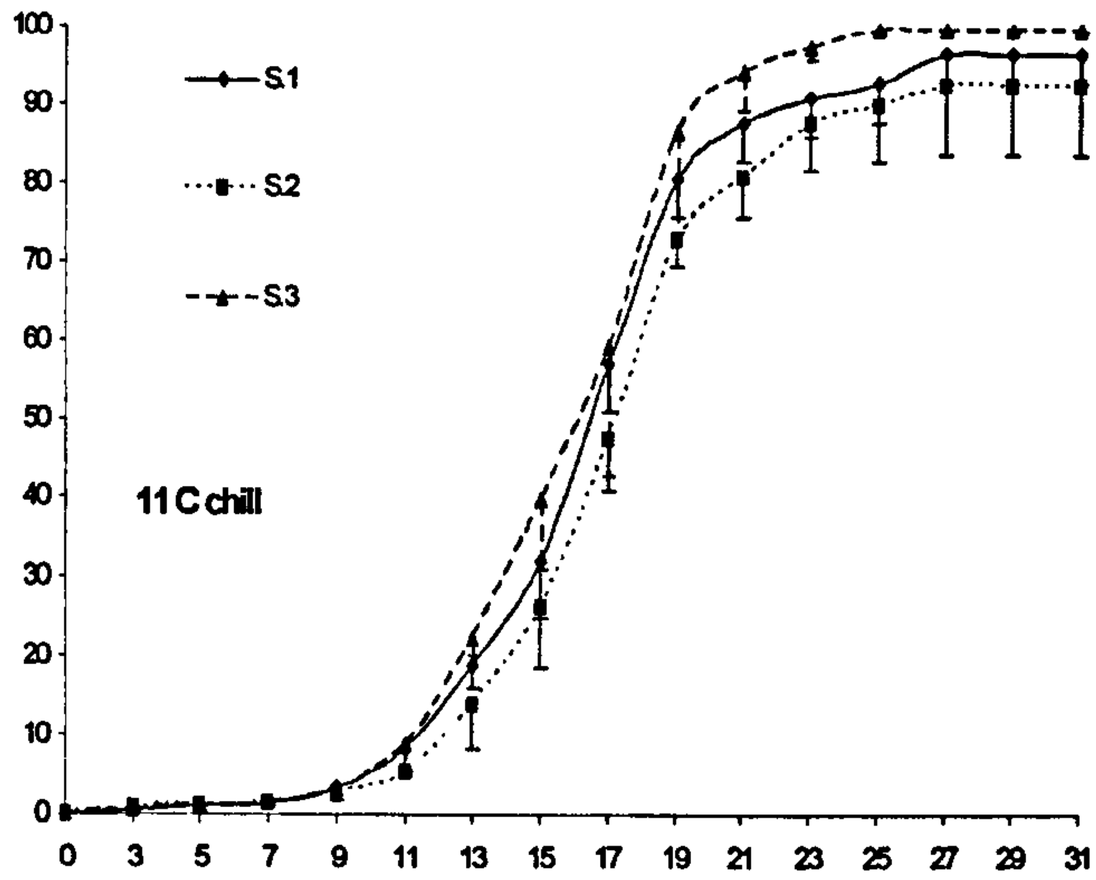
Apart from the occasional larva no hatched larvae were seen amongst the large numbers of eggs stored at 20°C. The observed cumulative proportions of hatching are illustrated in figure 5.2. In the non-chill treatments, the proportions hatch of isolate 3 were significantly higher than those of isolate 1 and 2 ($F_{2,35} = 262.37$, $p < 0.001$). Isolates 1 and 2 showed very similar proportions of hatch at each of the temperature settings ($p = 0.747$). Varying

Isolate	Mean L (µm)	Range	Mean W (µm)	Range	Mean V ($\mu m^3 * 10^5$)	SD (V) ($* 10^5$)
Amos	167.4	145.3-186.8	77.2	67.0-96.2	4.37	0.78
Scottish 1	156.6	136.8-186.1	72.7	61.8-91.3	5.49	0.92
Scottish 2	162.9	144.5-195.9	77.0	65.7-96.6	4.80	0.60
Scottish 3	161.6	139.2-193.2	75.2	66.4-86.4	5.28	1.09
Filicollis	155.3	140.2-180.9	91.5	80.2-109.3	6.87	1.23
Shorb, 1940 (*filicollis*)	169.6	148.2-195.4	87.0	74.4-106.7	6.94	1.58

Table 5.3 The length (L), width (W) and estimated volume (V) of 6 *Nematodirus* isolates. 'Shorb, 1940' represents the measurements of 71 *N. filicollis* eggs extracted from the paper by Shorb. For all isolates the $\pi/6 * LW^2$ volume results are given.

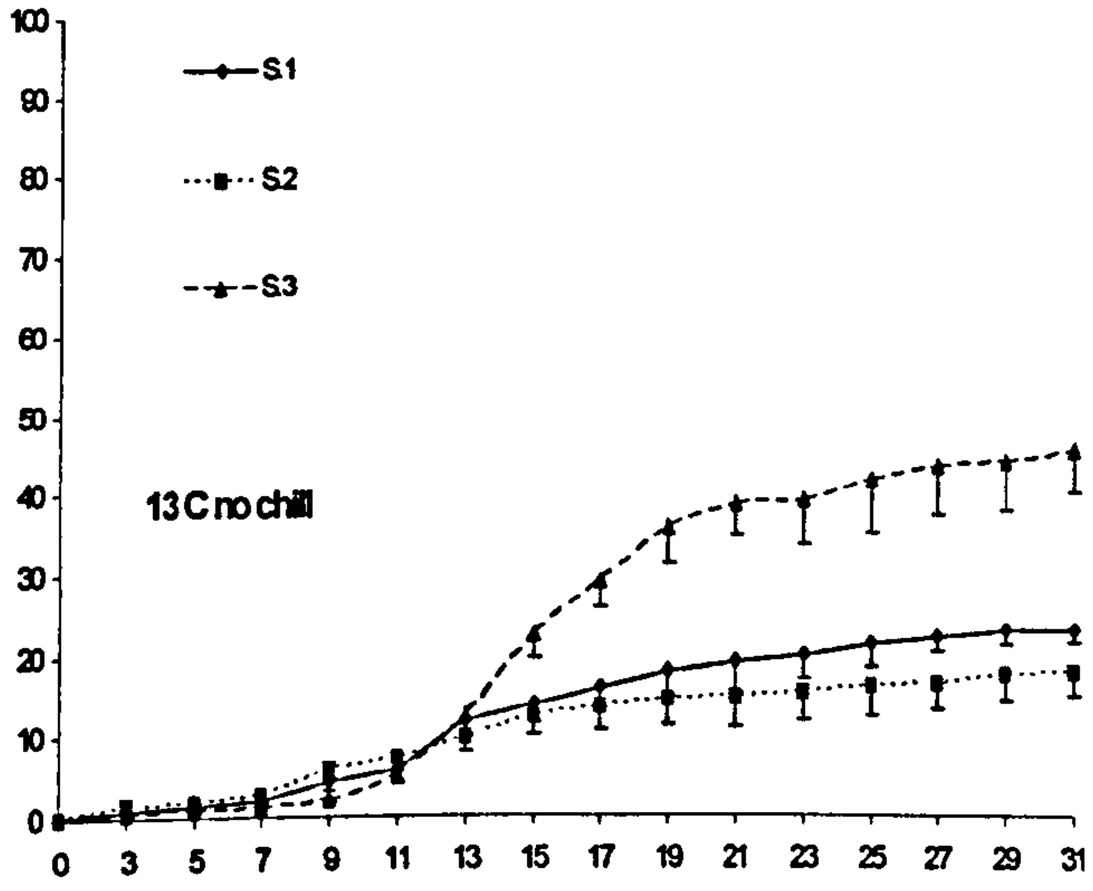
temperature settings also significantly influenced hatching patterns ($F_{3,35} = 16.59$, $p < 0.001$). At a temperature of 13°C more eggs hatched than at any other temperature ($p \leq 0.013$) while at 17°C fewer eggs hatched than in the other treatments ($p \leq 0.031$). When kept at 11°C, proportions hatch increased significantly between days 31 and 63 for isolates 1 and 3 ($t_2 = 6.62$, $p = 0.002$ and $t_2 = 4.82$, $p = 0.011$, respectively), but not for isolate 2 ($t_2 = 0.89$, $p = 0.477$). In the chill treatments the three isolates also showed significantly different proportions of hatch ($F_{2,35} = 15.00$, $p < 0.001$). However, in these treatments isolate 2 showed lower proportions of hatch than isolate 1 ($p = 0.004$) while the latter isolate was not separated from isolate 3 ($p = 0.214$). Again, increasing temperature decreased hatching proportions ($F_{3,35} = 4.05$, $p = 0.018$).

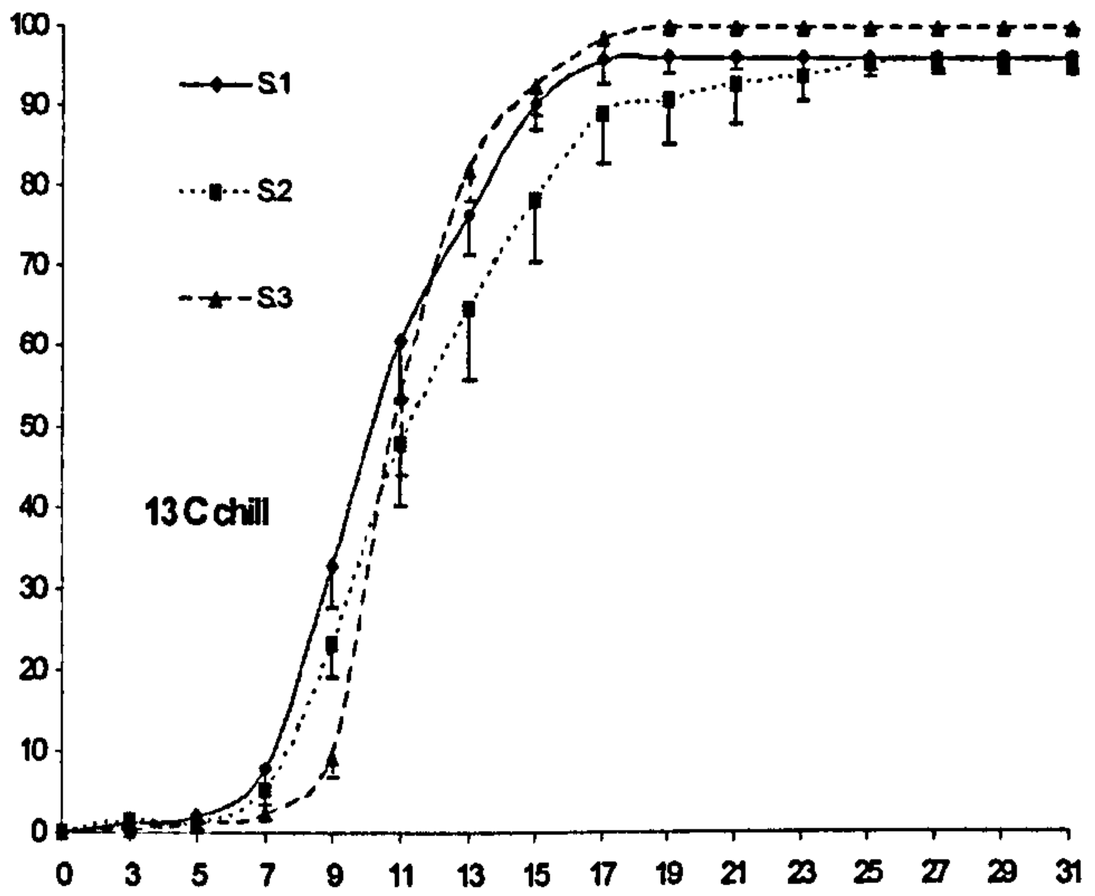


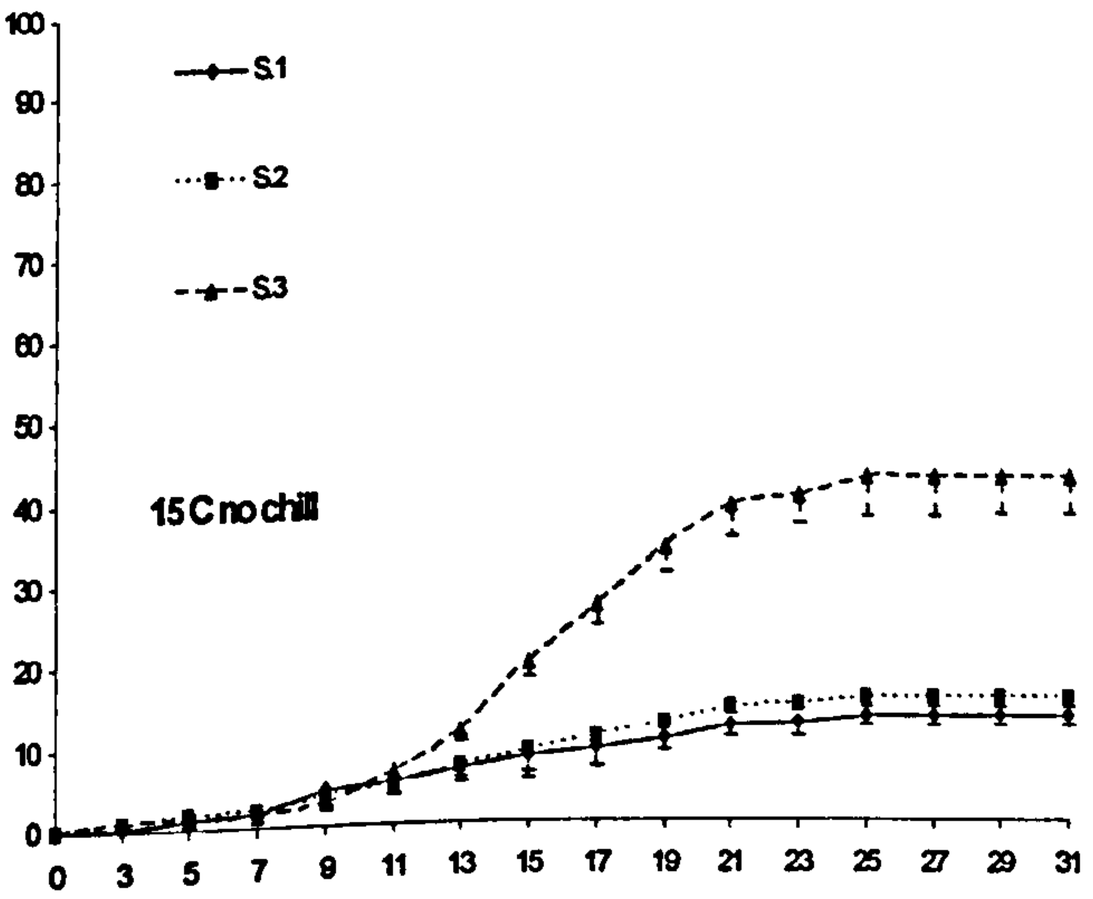


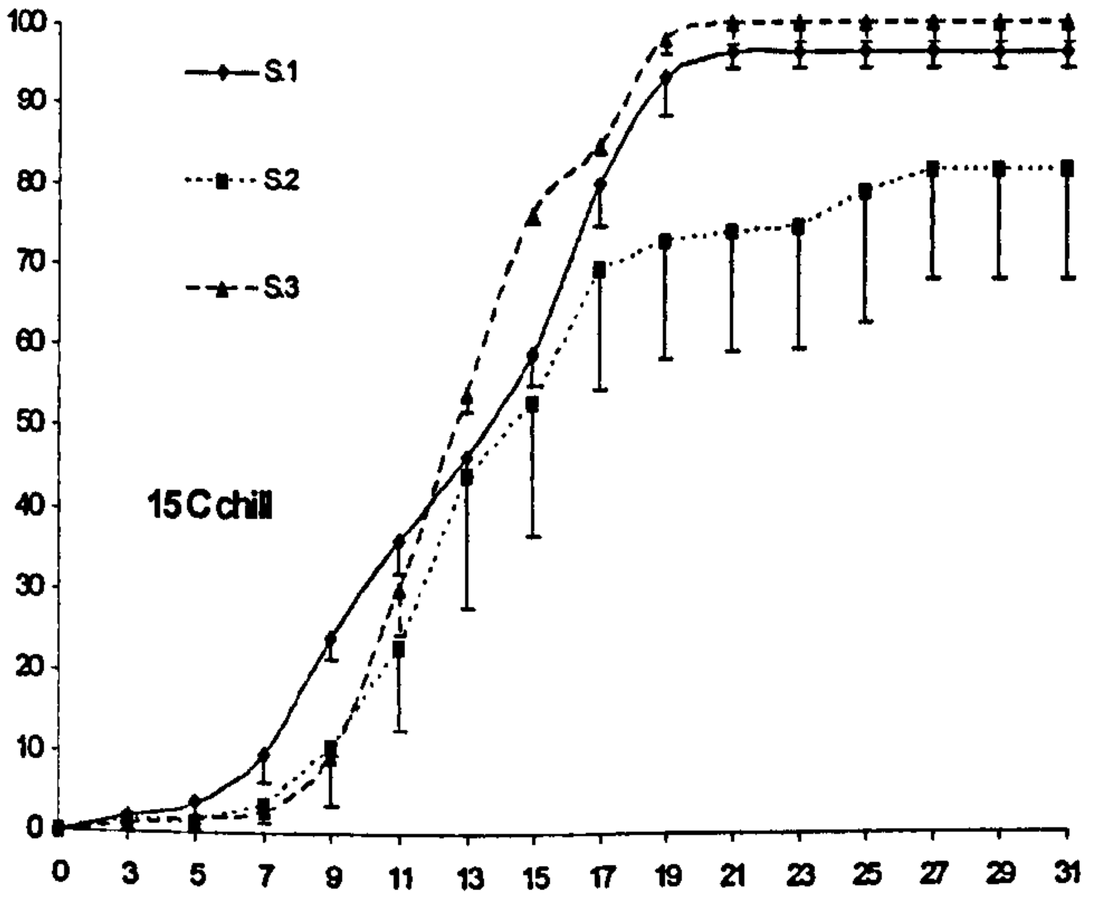
→ X-axes: days at hatching temperature

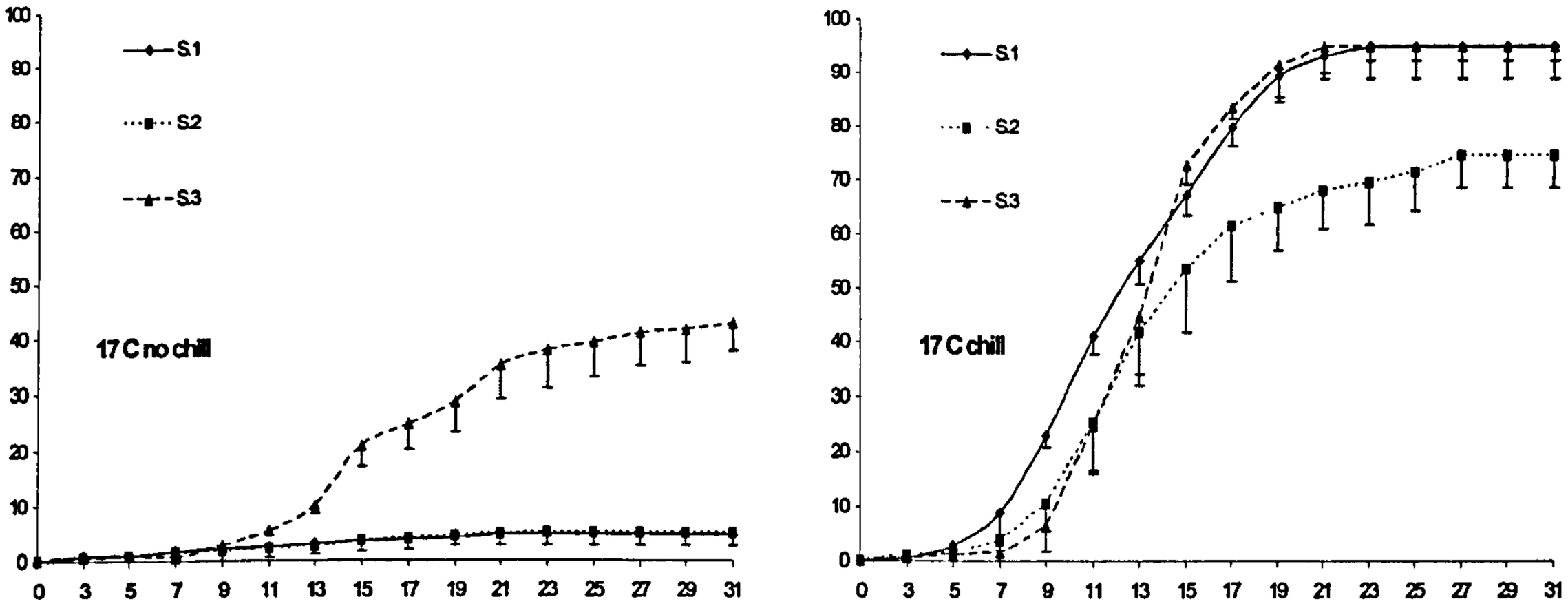
↑ Y-axes: cumulative percentage hatch











→ X-axes: days at hatching temperature
↑ Y-axes: cumulative percentage hatch

Figure 5.2 Cumulative proportions of hatching of the three Scottish isolates (S.1, S.2 and S.3) at various temperatures within the hatching range. On the horizontal axis the number of hatching days is given, on the vertical axis the cumulative proportions hatching.

At 11 and 13°C proportions were higher than at 17°C ($p \leq 0.029$) while the 15 and 17°C treatments were statistically similar ($p = 0.233$).

Differences in the timing of the completion of the initial 50% of the hatch were small but significant between isolates ($F_{2,71} = 28.80$, $p < 0.001$). Isolate 3 normally started to hatch later ($p < 0.001$) while isolates 1 and 2 responded to hatching stimuli on similar days ($p = 0.194$). In chilled treatments hatching started significantly earlier than in non-chilled treatments ($F_{1,71} = 36.41$, $p < 0.001$). At 11°C t_{50} occurred later than in all other treatments ($F_{3,71} = 76.51$, $p < 0.001$) while there was large overlap between the other three treatments ($p \geq 0.243$).

5.3.3 N. filicollis thresholds

Development

Results are shown in table 5.4. At 11.5°C very few eggs reached the L3 stage and therefore fresh eggs were put at 12°C, at which temperature all viable eggs managed to complete their development. At 30°C no development occurred at all. As was found in the previous chapter, arcsine transformed proportions of eggs developing to the L3 stage differed significantly between treatments ($F_{4,14}$= 48.53, p < 0.001). However, *N. fillicollis* eggs showed the reverse trend of that shown by *N. battus*: in the 12 and 13°C treatments significantly fewer (p < 0.001) eggs developed than in the 15, 20 and 25°C treatments while there were no differences between the latter three treatments (p ≥ 0.211). The optimum temperature for egg development, from these experiments, was therefore estimated at 25°C. The daily development rate to 50% egg development increased significantly with temperature (Pearson r_p= 0.97, p < 0.001) and is given in figure 5.2. Extrapolation of the regression line predicted the minimum development threshold to be 1.06 °C. As the confidence intervals of the slopes of this regression line and that of *N. battus* presented in chapter 4 do not overlap *N. filicollis* appears to complete its development significantly faster. There were no significant differences in the time taken between the onset and completion of development ($t_{min-max}$) between treatments ($F_{4,14}$= 0.575, p = 0.687).

T_s	T_m	D_{50}	D_{max}	$t_{min-max}$	DD_{50}	% L3
11.5	11.5 (10.9-12.5)	Incomplete development	-	-	-	-
12	12.1 (11.5-13.0)	50 (48-51)	54 (53-55)	4 (3-5)	548 (530-563)	55 (53-55)
13	13.4 (12.4-14.0)	48 (48-49)	52 (52)	4 (3-4)	595 (591-604)	55 (50-57)
15	14.9 (13.9-16.3)	47 (46-48)	52 (50-54)	5 (4-6)	652 (638-666)	83 (80-89)
20	20.7 (19.6-22.4)	27 (26-28)	31 (28-34)	4 (2-6)	538 (511-570)	84 (82-85)
25	25.2 (24.6-25.7)	25 (24-26)	29 (28-30)	4 (4-5)	604 (579-627)	79 (77-81)
30	30.0 (29.1-30.7)	No development	-	-	-	-

Table 5.4 *N. filicollis* egg development at various temperatures. T_s = temperature setting; T_m = mean measured temperature; D_{50} = average day 50% of the eggs had completed their development; D_{max} = average day development of all eggs was completed; $t_{min-max}$ = time between the detection of the first L3 and the completion of development (in days); DD_{50} = average number of Degree Days to 50% development; % L3 = percentage of the eggs present at the start of the experiment completing development. Presented percentages and days all rounded up to the nearest integer. The ranges are given between brackets.

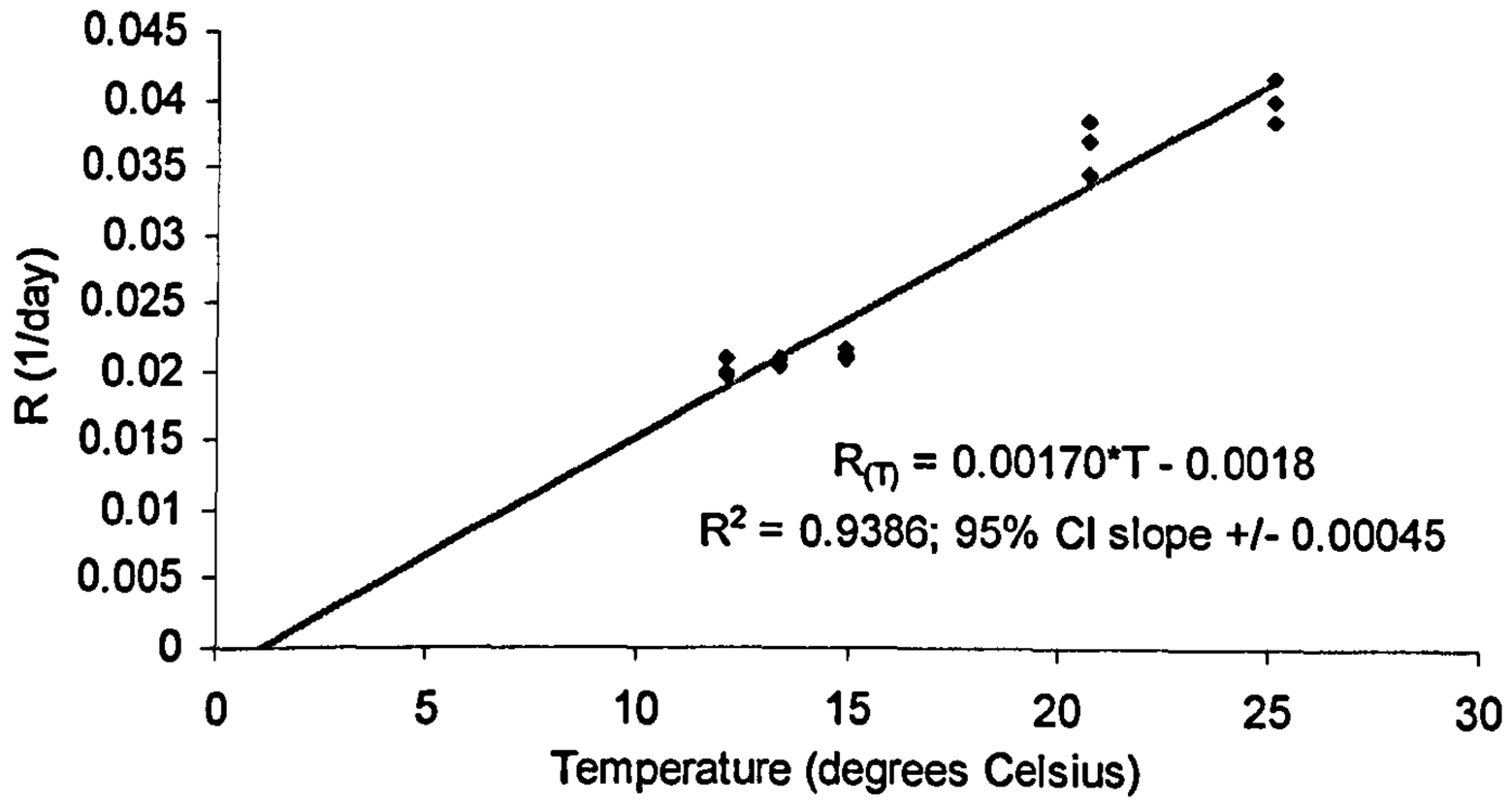


Figure 5.2 Regression of daily development rate (R) of *N. filicollis* on temperature. Please note that, as the result of overlap, not all 15 data points are visible.

Pre-hatch larval survival at 20°C

At the hatching temperature of 15°C, 98% (range 96-100) of the eggs hatched and hatching was completed on day 6.

Hatching

Temperature thresholds for hatching

Virtually no hatching was observed in the non-chilled treatments. After 29 days at the hatching temperatures the highest percentage hatch in any of the 30 replicas of the 10 non-chilled treatments, at a temperature of 13°C, was 3.5%. In most replicas no hatching was observed at all.

T_s	T_m	% hatch	t_{50}
4	4.3 (3.7-5.1)	8 (7-9)	19 (18-20)
6	6.0 (4.6-6.6)	11 (10-13)	4 (4-5)
9	8.8 (8.4-9.3)	24 (22-27)	3 (3-2)
11	10.8 (10.2-11.1)	53 (50-55)	2 (1-2)
13	13.1 (12.7-14.2)	72 (67-79)	1 (1-2)
15	14.9 (13.9-15.3)	27 (25-30)	3 (2-3)
17	17.1 (16.5-17.5)	18 (16-22)	4 (4-5)
20	19.7 (19.2-20.9)	1 (0-2)	-
7-13	6.8- 13.2 (5.8-7.7; 12.1-14.0)	44 (41-48)	5 (5-6)
14-20	14.6- 19.8 (14.2-14.9; 19.1-20.9)	77 (72-82)	1(1-2)

Table 5.5 Hatching of *N.filicollis* eggs at various temperatures. T_s = temperature setting; T_m = mean measured temperature; % hatch = mean cumulative percentage hatching in the chilling treatments; t_{50} = mean time (in days) to 50% hatch All measurements are rounded up to the nearest integer. Ranges are given between brackets.

The hatching characteristics of the chilled treatments are summarised in table 5.5.

As virtually no eggs hatched at 20°C this treatment was omitted from further analyses.

Hatching percentages were significantly different between treatments ($F_{8,26}$ = 119.82, $p < 0.001$). At constant temperatures, at 9°C more eggs hatched than at 4 and 6°C ($p \leq 0.036$) while at each further temperature increase, up to 13°C, more eggs hatched ($p < 0.001$). At temperatures higher than 13°C hatching percentages decreased again ($p < 0.001$) with each 2°C increase. Hatching percentages of the fluctuating 7-13°C and 11°C treatments ($p = 0.192$), and fluctuating 14-20°C and 13 °C treatment ($p = 0.655$), could not be statistically separated.

Figure 5.3 illustrates hatching at 11-17°C. At temperatures optimal for hatching, large proportions of eggs hatched on day 1-3 and hatching was completed within one week.

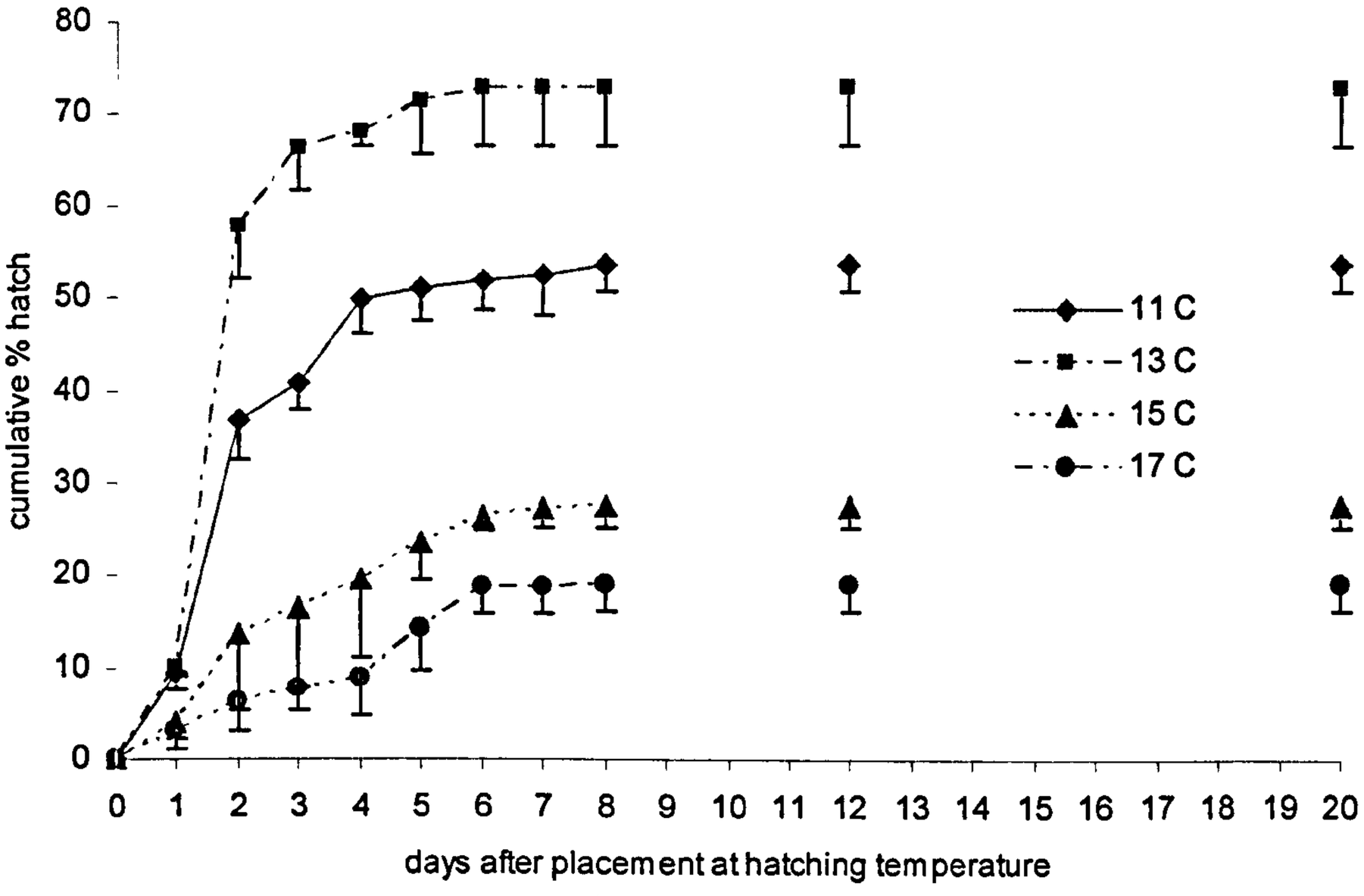


Figure 5.3 The hatching of chilled *N. filicollis* eggs at 11,13,15 and 17°C.

The t_{50} of the 4°C treatment was significantly longer than that of all other treatments ($F_{8,26}$ = 215.06 , $p < 0.001$) but otherwise no clear patterns could be established in the time taken to 50% hatch as there were large overlaps between homogeneous subsets of treatments.

Temperature threshold for chilling experience

After placement at 13°C, on hatching day 8, 52% (49-56) and 38% (36-45) of the 6°C and 8°C treatments had hatched, respectively. However, in the 11°C treatment a hatch of only 3% (1-4) of the larvae was recorded. The 11°C treatment was subsequently kept at 11°C for a further 6 weeks and the process repeated but no further hatching was observed.

Survival

The instantaneous daily mortality rates of L3 are given in table 5.6. Larval mortality rates were significantly lower at 9 and 11°C than at all other temperatures. Within the hatching range, larvae survived significantly longer at 13 than at 17°C. Compared to all temperatures at which hatching was observed, mortality rates increased very significantly at 20°C. At fluctuating 14-20°C survival could not be separated from both 20 and 25°C treatments.

Temp. (°C)	9	11	13	15	17	20	25	30	14-20
μ	0.0012 (0.00028)	0.0012 (0.00050)	0.0020 (0.00023)	0.0021 (0.00025)	0.0026 (0.00032)	0.0161 (0.0025)	0.025 (0.0037)	0.039 (0.0095)	0.020 (0.0051)

Table 5.6 Survival of chilled *N. filicollis* larvae. μ = the slope of the log linear regression line and equals the instantaneous daily mortality rate; 95% confidence intervals (plus or minus) are given between brackets.

5.4 Discussion

The three *N.battus* field isolates were taken from one region. Each had caused clinical disease in lambs in spring and would therefore be presumed to be biased towards a spring hatch of chilled eggs. However, all three also expressed hatching of non-chilled eggs and the degree to which these eggs hatched varied significantly between isolates. The presented work therefore confirms that the hatching of non-chilled eggs is a trait expressed by *Nematodirus battus* and shows that the degree to which isolates express this trait varies.

From these results it cannot be determined whether differences in proportions non-chill hatch are the result of random between-isolate variation or genetic selection or phenotypic plasticity. Either way, from the work presented in chapter 2 it would be hypothesised that Scottish *N. battus* is under less climatic pressure to hatch without chilling. These isolates did not hatch in the same incubators, at the same time, as the Bristol isolate and therefore the four isolates cannot be statistically compared. However, it clearly appears that larger proportions of non-chilled eggs of the Bristol isolate hatched than in any of the three Scottish isolates.

No obvious differences in grazing management could be identified on the three Scottish farms. As it is the only isolate from a farm located in an elevated position, on a hillside, it is tempting to speculate that the higher proportions of non-chilled larval hatch found on farm 3 may be the result of greater climate uncertainty. Working at pasture, Thomas (1991) showed that upland and lowland *N. battus* strains may indeed show very different hatching behaviour.

Although the *N.filicollis* culture was not 'pure' 95% of eggs were identified as belonging to the species. In hatching experiments the 5% *N. battus* eggs did not interfere with the main periods of hatching as hatching of *N. filicollis* eggs is completed far more rapidly. During the hatching experiments *N. battus* larvae, which can easily be distinguished from *N. filicollis* larvae even at low magnifications, were only ever seen towards the tail end of the experiments, in frequencies matching those found in the egg morphology experiment, and excluded from the counts.

Differences between isolates were not limited to those found in the proportions hatch of non-chilled eggs. Two isolates showed little decrease in proportions hatch of chilled eggs towards the upper temperature threshold while a third, as the Bristol isolate, clearly did. Interestingly, the *N. filicollis* isolate from Bristol also expressed this but to a far greater extent than any of the four *N. battus* isolates. Also, isolates behaved like one isolate in the non-chilled treatments but as a different one in the chilled treatments. For example, isolate 1 behaved like isolate 2 in the non-chill treatments but like isolate 3 in the chill treatments. It may be that each *N. battus* isolate has its own fine-tuned hatching characteristics. The hatching response of *Nematodirus* species to temperature stimuli within the temperature thresholds would then seem to be highly adaptable. However, the results may also reflect random between-isolate variation.

The presence of an upper threshold for hatching, approximating 17°C, was confirmed, not only for the *N. battus* isolates but also for the *N. filicollis* isolate. It is likely that this upper temperature threshold plays a far more important, limiting, role in *N. battus* than in *N. filicollis* epidemiology. First, *N. filicollis* eggs start to hatch at lower temperatures and therefore hatches over a larger temperature range. At the temperatures hatching commences (approximately 6°C), under UK climatic conditions, the maximum daily temperature is unlikely to be above the upper threshold. Second, hatching is completed much faster: in as little as two days very large proportions of the population may have hatched and hatching is completed on day six. As the eggs have to take up water during the build-up to the hatch (Parkin,1972) it may be that *N. filicollis* egg shells are able to change their permeability for water at a faster rate. As a result, the maximum and minimum temperature only has to

remain within the thresholds for very few days, as opposed to 16-20 days for *N. battus*. In the UK, and especially in warmer climates, the probability of an egg hatching in a given year would be predicted to be much greater for *N. filicollis* than for *N. battus*.

The lower hatching threshold of *N.filicollis*, in combination with a gradual release of more larvae as the temperature increases up to 13°C, can explain its between-year differences in hatching patterns observed at pasture as well as an apparent lower pathogenicity of the species in the UK. If temperature rises occur quickly a mass hatch may occur (as observed by Thomas, 1959b; Gibson,1963; Boag and Thomas,1975) but more frequently, in winter or early spring, temperature will rise within the lower threshold for a couple of days, then fall below it again. For *N. battus* no hatching will result but for *N. filicollis* this is likely to trigger a gradual hatch of the depot of eggs and thus a more gradual build-up of immunity in exposed sheep.

N. filicollis egg development starts at a temperature threshold similar to that of *N. battus* but development is quicker while, in contrast to *N. battus*, more eggs develop towards the higher end of the development range. Also, unlike *N.battus*, towards the upper end of the development threshold development still takes place in the characteristic highly synchronised manner. Thus, the developmental phase of *N.filicollis* appears to be better adapted to warmer climates. However, as the hatching of these eggs is bound by constraints very similar to those of *N. battus*, this advantage does not pay off in any way in the UK or in more tropical climates. Very few eggs died at 12 weeks of constant 20°C but *N. battus* eggs were also highly resistant to prolonged temperatures up to 25°C. Although *N. filicollis* appears to be able to withstand a constant temperature of 30°C better than *N. battus*, death

rates of the two species at all other temperatures were identical. It appears that the only thing setting the free-living stages of two species apart is the difference in lower threshold and the faster hatch within the temperature threshold, i.e. the probability of hatching.

The autumn peaks in *N. filicollis* larval emergence, followed by larger peaks in February-April, described by Boag and Thomas (1975) can only be explained by the hatching of non-chilled eggs. Gibson (1959b) described mass hatching in spring, similar to that of *N. battus*, but also the hatching of L3 8 weeks after the deposition of unembryonated eggs at pasture. Gibson and Everett (1976b) even recorded mass larval emergence of larvae anywhere between June and November, depending on when, in the same calendar year, unembryonated eggs had been put at pasture. This shows that virtually all eggs hatched on the completion of development, without any chilling experience. Alongside *N. spathiger* and *N. helvetianus*, *N. filicollis* can be found all over the world and is prominently present in ruminant habitants of countries with very hot climates indeed. The hatching strategy would thus be expected to bear closer resemblance to those of *N. spathiger* and *N. helvetianus*, species readily hatching on the completion of development of L3, than that of *N. battus*. In genetic studies *N. spathiger* and *N. helvetianus* were repeatedly identified as the closest related sister species, but with these two forming a clade with *N. filicollis*. *N. battus* was shown to be by far the most genetically distinct species (Newton *et al.*1998; Audebert *et al.* 2000; Nadler *et al.* 2000). Although these genetic differences do not have to be reflected in the hatching behaviour, they were expected to, in some way, reflect the phenotypical characteristics arguably setting them apart the most. The hatching of *N. filicollis* eggs after chilling only was therefore highly unexpected. Given the results at pasture cited above and the persistence

of the species in countries like Iraq, the finding in itself once again strongly supports the hypothesis that , in *Nematodirus* species, the proportion of eggs hatching without chilling is highly adaptable.

On the farm near Bristol *N. filicollis* was isolated from, worm eggs present in the dung of lambs were studied throughout the year, for two years (see chapter 7), but indeed increased egg counts of this species were not witnessed in autumn. The persistence of two *Nematodirus* species on one locality, both apparently capable of hatching without the need for chilling but only one species doing so, may underline the importance of host immunity, and the interaction of parasite species, in co-shaping the direction of the adaptation of hatching behaviour.

As on the farm described by Helle (1969), *N. battus* was clearly the dominant species, only very few *N. filicollis* ever being detected in the dung of groups of animals outside of the one recorded peak. In order to have a constant supply of organic meat for local markets the farm uses Dorset Poll sheep and is somewhat unique in having three lamb crops a year, one group of ewes lambing in December, one in February and one in April. The crops of lambs are circulated over 8 plots of pasture and this system has been run for the past 12 years. The February and, to a lesser extent, April groups showed *N. battus* egg counts mirroring the spring peak of larval emergence in both years. The *N. filicollis* peak was only registered in the December crop of lambs. Given the lower hatching thresholds of the species the main hatch would indeed be predicted to occur during the mild winters and, perhaps, very early spring. February-born lambs only start to consume significant amounts of herbage in April. At this time, not only would a large proportion of *N. filicollis* larvae have died, the *N. battus*

peak would also be likely to occur. At the very high densities of *Nematodirus* worms present in the host during spring peaks any *N.filicollis* larvae consumed would be expected to suffer the effects of crowding (Keymer, 1982; Boag *et al.* 2001) or a strong immune response (Paterson and Viney, 2002). Similarly, as the upper thresholds are very similar, autumn hatches of the two species would occur virtually simultaneously. Larger numbers of *N. battus* would hatch while the hosts, at this time of the year, would already have a degree of immunity to *Nematodirus* infection. As February and March seem the only months *Nematodirus*-naive young hosts are around on the farm, the hatching of *N.filicollis* after chilling only could therefore be interpreted as a strategy to avoid both co-infection with *N. battus* and the infection of immuno-competent hosts.

As was found for *N. battus*, the predicted *N. filicollis* development threshold (1.06°C) bears little resemblance to reality, supporting suspicions surrounding the use of these techniques for arctic parasites. Once again surprisingly for a parasite persisting in hot climates, the threshold for chilling experience of *N. filicollis* appears to be lower than that of *N. battus*. Two *N. battus* isolates, like the Bristol isolate, apparently managed to complete a chilling process at 11°C while isolate 2 did not. As, in all isolates, the same enzymes will be involved in the chilling processes this cannot easily be explained.

The egg volumes of two *N.battus* isolates were significantly smaller than those of two others. Phenotypic plasticity would be expected to give a very large variance for each isolate but perhaps no significant differences between isolates. Therefore, the results may suggest that either the genetically determined egg size, or the degree to which the genome codes for

variability in eggs size, differs between isolates. However, fecundity, and perhaps egg size, may also be influenced by co-infection with other parasites (Mapes and Coop, 1973), a factor not controlled for here.

N. filicollis egg volumes may be somewhat larger than those of *N.battus* but this is sensitive to the formula applied for the calculation of egg volume. However, even if egg volumes of the two species are very similar, it is puzzling to see that the larvae of *N. fillicollis* are smaller than those of *N.battus*. In a fully embryonated *N. battus* egg the very tightly rolled-up larva completely fills the egg. In the *N. filicollis* egg surplus space can be observed around the larva, and the production of eggs larger than strictly necessary would be predicted to result in sub-optimal fecundity. This may suggest that the egg shell actually represents a low energy investment to the female worm in comparison to the embryonic material inside the egg. If this is true, in the study of the evolution of energy investment in offspring, larvae would have to be measured instead of eggs. It is not known whether there are significant differences in fecundity between *N.battus* and *N. filicollis*.

Tetley (1941) measured 280 *N. filicollis* eggs and reported measurement ranges of 133-176 µm (L) and 71-84 µm (W). Thomas (1559a) measured 100 eggs and reported lengths in the range of 134-168 µm and widths of 71-78 µm. Thomas also measured 100 *N. battus* eggs with lengths of 152-182 µm and widths of 67-77µm. Parkin (1972) reported mean lengths of 160 µm and mean widths of 70 µm for this species. These results have in common with those presented by Shorb (1940) that they all represent eggs less wide than those measured in this study. As the width of the eggs has the largest effect on the volume, this would suggest that egg volume has increased rather than decreased in recent decennia. The results

from the studies also have in common that their range is much narrower than those of the field isolates presented here. This may suggest an increase in coding for variability in egg size.

In nematodirae, the enzymes transforming lipid reserves into carbohydrate reserves (Ash and Atkinson, 1983, 1986) appear to only be able to do their work below the minimum temperature threshold for 'chilling'. The eggs of *N. spathiger* (Tetley, 1941) and *N. helvetianus* (Herlich, 1954) are very significantly larger than those of *N. battus* and *N. filicollis*. Increased egg size, and higher volumes of lipid reserves per larva, could therefore be interpreted as a compensation for the lower quality of the energy reserves. Egg populations hatching a higher proportion of larvae as non-chilled larvae would then be expected to be larger. In this study, egg size could not be related to the hatching strategy.

5.5 Conclusions

Nematodirae are very well adapted to extreme environments but, in warmer climates, the key to their transmission success lies in the probability of the completion of the hatching process. As a result of the upper threshold for hatching, variability in hatching behaviour is likely to play an integral part in the ecology of their free-living stages. However, hatching characteristics are likely to be driven not only by climatic factors but also by host availability and, perhaps, interspecific interaction. As a result, proportions of egg populations hatching without chilling may vary considerably both between regions and at much smaller spatial scales.

The variability in the proportions of *N.battus* eggs hatching without chilling poses a challenge to the mathematical modelling of the ecology of *N. battus*. Chapter 7 will explicitly explore the effects of varying the hatchability of non-chilled eggs in different climatic environments further.

Chapter 6 - The influence of water on the hatching and migration of infective larvae

6.1 Introduction

Many studies have reported a positive correlation between rainfall and the emergence of trichostrongyloid larvae on herbage or the increase of worm burdens in tracer animals (e.g. Williams and Bilkovich, 1973; Bryan and Kerr, 1989; Stromberg, 1997). Not surprisingly, such associations are most dramatic in semi-arid regions, where absence of rain may bring parasite transmission to a halt during the driest months and sharp peaks of larval emergence can be seen after periods of rainfall (Chiejina and Fakae, 1989; Onyali *et al.* 1990; Agyei, 1997; Sissay *et al.* 2007). Water may play a role at several crucial stages in the life cycles of trichostrongyloids, noticeably the incorporation of *Nematodirus* eggs into the soil, the development, and probably hatching, of eggs, prevention of desiccation of eggs and hatched larvae, and the migration of infective larvae away from the faeces and onto the herbage.

6.1.1. Incorporation of eggs into the soil

As shown by Gibson and Everett (1981), and by the pasture experiment described in chapter 4, *Nematodirus* eggs do not develop in dung and may die if captured in dung for prolonged periods. As, in the field experiments, very little visible degradation of desiccated dung occurred this process is likely to be strongly influenced by rainfall. In chapter 7 the likely delaying effect of egg incorporation into soil on the onset of egg development in the UK, as well as possible differences between regions, are explored in a simple model.

6.1.2. Development of eggs

The experiments of Parkin (1976) clearly showed that, regardless of high or low relative air humidity, *Nematodirus battus* eggs which were deprived of free water did not manage to develop. However, eggs develop in the soil and soil particles are surrounded by a layer of water, which is retained by forces of capillarity, osmotic pressure and gravity (Parkin,1975b). At osmotic pressures reflecting soil saturations between full water capacity and wilting point, *N. battus* eggs are still able to take up water (Parkin, 1975b). Only eggs incubated in molar solutions are not able to develop beyond the 2nd larval stage while those incubated in salt solutions of strengths between 10^{-4} and 0.1M manage to develop just as well as aqueous controls (Parkin, 1976). The development of *Trichostrongylus* spp. and *Haemonchus contortus* has been shown to cease only during summers of 2-3 months drought accompanied by relative humidities as low as 20% and maximum mean monthly temperatures approximating 32°C (Onyali *et al.* 1990; Garcia Romero *et al.* 1997). On warm spring and summer days, these species manage to reach the L3 stage even in dung which becomes very desiccated before development is completed (Chiejina and Fakae, 1989; own observations at pasture). In temperate regions, access to water is therefore unlikely to be limiting to the development of trichostrongyloid species in general, or to *Nematodirus* species in particular.

6.1.3. Desiccation of eggs and larvae

The eggs and larvae of Nematodirae are remarkably resistant to desiccated environments. Further to the development of unembryonated eggs in the salt solutions mentioned above,

Parkin (1976) reported no appreciable mortality after keeping air-dried embryonated eggs at a relative humidity of 33% for 19 weeks. Only approximately one quarter of hatched infective larvae died as the result of this treatment when applied for 10 weeks (Parkin, 1972). Zurliiski (1978, as quoted by Anderson, 2000), who dried the larvae of *N. spathiger* at 15-20°C for 166 days, confirmed that such desiccated larvae are still infective to lambs.

6.1.4. Hatching of eggs

Anecdotal evidence suggests that when a period of rain follows a prolonged period of drought, the prevalence of nematodirosis may increase. Therefore, amongst UK veterinarians, it is popular belief that rain can induce hatching. For example, during the spring of 2003, a year in which a relatively high disease incidence was recorded, the temperature rose above the lower threshold for hatching in April but hardly any disease was witnessed during this very dry month (VIDA data). The peak incidence of disease was measured towards the end of May, just after rains ended a prolonged period of drought. Similar circumstances occurred during the very dry spring of 2007, in which no peak of larval emergence was measured at pasture until the start of the very wet month of July (see chapter 7). If the hatching of eggs when water becomes available after a period of desiccation indeed represents an alternative mechanism to the described temperature-induced hatching this phenomenon will have to be included in mathematical models of the epidemiology of nematodirosis. As the result of climate change, periods of drought, followed by a limited number of days with heavy rainfall, have been predicted to become more frequent (Hennessy *et al.* 1997; Tapiador *et al.* 2007).

A first working hypothesis would be that, during droughts, water becomes limiting for the hatching of eggs that have been temperature-primed to do so. However, Parkin (1975b), reproducing soil moisture stresses at the level observed at wilting point during droughts, not only observed the hatching of eggs submitted to this treatment but also suggested that, after heavy rains, the aeration of the soil becomes the limiting factor for hatching. In further experiments Parkin (1976) observed the hatching of 'chilled' embryonated eggs in 0.1-10^{-4} M salt solutions. All this suggests that soil moisture is unlikely to be limiting for the hatching process and also that the presence of water is no guarantee of hatching.

A second hypothesis would be that desiccated eggs, under the influence of rapid influx of water when it becomes available once more, hatch as the result of the suddenly increased hydrostatic pressure. Increases in water content have been shown to take place in larvae immediately prior to hatching (Perry,1977; Perry, 1989). Parkin (1976) incubated 'chilled', air dried, eggs above salt solutions representing certain lowered relative humidities (RH) and found, on return to water, a somewhat earlier and more extensive hatch of eggs which had been exposed to lowered humidities compared with aqueous controls. However, a 100% RH treatment (air dried eggs kept above distilled water), on return to water, hatched more rapidly than any of the other treatments, casting doubt on Parkin's conclusion that exposure to lowered humidity significantly increased hatching magnitude and rate. Eggs in the soil are likely to experience high osmotic pressures as a result of surrounding soil particles and salts and thus the moisture stresses experienced in the soil may be far greater than those represented by lowered air humidity alone (Parkin, 1975b). Also, Parkin worked with chilled eggs only. It has been observed that the nematode *Aphelenchus avenae* rapidly changes its

lipid reserves into carbohydrate reserves when undergoing desiccation (Madin and Crowe, 1975), suggesting that increased osmotic pressure caused by an increase in carbohydrates may play a role in protection from desiccation. Therefore, chilled eggs may be more resistant to desiccation and dehydration-rehydration cycles may have different influences on the hatching of chilled and non-chilled eggs.

The aim of the laboratory experiments on this subject is to establish whether

(i) rehydration of desiccated *N. battus* eggs leads to temporally altered hatching patterns;

(ii) hatching induced by rehydration would be likely to free enough larvae to cause clinical disease;

(iii) chilled eggs are better protected against desiccation.

6.1.5. Migration of hatched larvae

The migration of the infective larvae of most trichostrongyloids onto herbage can be split into at least two phases: a) migration out of dung and b) migration onto herbage. The latter phase may be sub-divided into lateral migration on soil/dead vegetation and vertical movement onto herbage. As the larvae of *Nematodirus* spp. hatch in the soil only phase b) applies to them.

The amount of specific research conducted into the migration of larvae of gastrointestinal nematodes, amounting to a handful of papers, is in sharp contrast with that published on the development of their free-living stages, which amounts to hundreds of papers. No specific

work on the migration of *Nematodirus* larvae has been published. The seminal work on the migration of infective larvae freed from dung was carried out on the species *Trichostrongylus retortaeformis* (Crofton, 1948), a parasite of rabbits, and *Haemonchus contortus* (Rees, 1950), and it is not clear whether migration behaviour is consistent between species and therefore whether the conclusions of these studies can be directly applied to *Nematodirus battus*. Also, studies investigating migration at pasture (Skinner and Todd, 1980; Rose and Small, 1985; Krecek *et al.* 1992; Marley *et al.* 2006) have put out eggs or larvae in dung, without paying attention to separate migration phases, and therefore it is not clear to which part of the migration phase the described effects apply. Finally, chapter 3 suggested that, for the species *T. circumcinta*, *T. colubriformis* and *H. contortus*, in the UK, temperature is the all-important driver behind both annual disease incidence and seasonal patterns in the occurrence of clinical disease. A study on the phase of the life cycle thought to be directly dependent on the presence of free water tests this hypothesis. For these reasons, while working towards a mathematical model of *N.battus* ecology, it seems appropriate to include the migration behaviour of other trichostrongyloids into a detailed investigation of the likely effects of rainfall on migration.

a) Migration out of dung

For *Ostertagia, Haemonchus* and *Cooperia* spp. present in the dung of cattle it has been shown that, once development is completed, L3 cannot escape from desiccated dung (Williams and Bilkovich, 1973; Chiejina and Fakae, 1989; Agyei, 1997). On dry, warm, days cattle faeces, which are normally more liquid than sheep faeces and dropped in pats

rather than in pellets, rapidly form a crust on the pat surface and it appears this crust, although it may protect them from further severe desiccation, cannot be penetrated by L3. In the tropics, the sudden migration of these larvae, made possible by the arrival of the rain season, may result in very sharp and dangerous peaks of larval emergence (Fakae and Chiejina, 1988; Chiejina and Fakae, 1989; Agyei, 1997). In the temperate regions it was also documented that drought-breaking rains suddenly increased larval density on herbage 10-fold as larvae, which had been trapped for months, migrated *en masse* (Bryan and Kerr, 1989). Cattle faeces can clearly function as a reservoir of larvae during drought. Sheep faeces have a different consistency and the small pellets are less likely to protect larvae from desiccation. Whether moisture is essential for the migration of larvae out of sheep faeces is not known. Skinner and Todd (1980) published the only study measuring larval presence in sheep pellets as well as on herbage and, although not backing up their statement with the data presented, suggested that larvae stayed in the pellets when there was no rain.

Estimations of larval death rates in desiccated dung have not been published.

b) Migration onto herbage

Several pasture studies (Skinner and Todd, 1980; Krecek *et al.* 1990; Niven *et al.* 2002) have suggested that a film of moisture is needed for the migration of larvae onto herbage, and this has become the established view amongst veterinary parasitologists. However, as continuous films of moisture are rarely witnessed on grass blades, and water may evaporate rapidly on summer days, this raises the question of whether larval migration is therefore only possible

either just after rains or when dew is found on grass leaves (Langrova *et al.* 2003). If it is indeed free water that determines whether larvae climb or not then rainfall patterns may show strong correlations with larvae emergence onto herbage and it would be wrong to ignore them in computer models predicting peaks of larval emergence. Silangwa and Todd (1964) compared migration onto wetted and non-wetted grass in the laboratory and concluded that trichostrongylid larvae climbed wetted grass significantly better. However, the experiments took place in humidity chambers at RH 80-90%. Therefore, the RH around the wetted grass may simply have been higher and this may have supported migration. Indeed, Callinan and Westcott (1986) produced similar results by varying only RH, suggesting that free water is not the driver of larval migration. Crofton (1948) and Rees (1950) suggested that gradients in relative humidity, and temperature, experienced by the larvae determined their migratory behavior. However, the work presented by these authors took place outdoors and rainfall and dew were not recorded. Therefore, as relative humidity itself will be correlated to rainfall, the question remains whether it is the presence of free water or a certain relative humidity that is needed for larval migration.

Studies on the larval migration of gastrointestinal nematodes (Crofton, 1948; Rees, 1950; Silangwa and Todd, 1964; Callinan and Westcott, 1986; Langrova *et al.* 2003) show remarkable consistency in the proportions of larvae recovered from herbage. Unfortunately, no study has quantified the sensitivity of the recovery method and thus true migration proportions cannot be estimated. However, it transpires that the vast majority of larvae may not make it onto the herbage. This raises the question of whether they form a larval reservoir in the soil from which individuals emerge over a prolonged period of time, or whether larvae

that remain in the soil perish. Longitudinal studies of larval emergence have not been undertaken.

Sturrock (1965) investigated the influence of heavy rains on the wash-out of *T. axei* larvae from the herbage and top layer of the soil, into deeper soil layers. It was found that, after heavy rains followed by artificial watering of herbage, approximately 85% of larvae was still recovered in the top 5cm of the soil while only 0.8% reached a depth of 25-30cm. Rose and Small (1985) confirmed the ineffectiveness of rainfall in washing *T. vitrinus* larvae into the soil. In contrast to popular belief, losses of larvae as the result of heavy rainfall thus appear to be very limited.

The aims of the present study in respect of larval migration are to establish:

(i) whether larvae can migrate from desiccated sheep dung;

(ii) whether the presence of free water is essential for the vertical migration of larvae;

(iii) the fate of larvae that initially remain in the soil.

6.2 Materials and methods

6.2.1 The influence of re-hydration on the hatching process

Bristol *N.battus* eggs were isolated as described in chapter 4 and incubated at 20°C for seven weeks. After thorough mixing, half of these eggs were 'chilled' at 4°C while the other half were salt-treated. After 4 weeks at 4°C, chilled eggs were also transferred to, and

Salt	Formula	Relative Humidity
Potassium nitrate	KNO_3	95%
Ammonium nitrate	NH_4NO_3	70%
Magnesium nitrate	$Mg(NO_3)_26H_2O$	55%
Magnesium chloride	$MgCl_26H_2O$	33%

Table 6.1: Salt solutions used in the desiccation experiments.

mixed with, saturated solutions (containing an excess of solids) of the salts given in table 6.1, representing, at 20°C, relative humidities of 95, 70, 55 and 33%. These salts were chosen for their lack of toxic effects (Winston and Bates, 1960; Parkin, 1976).The egg-containing salt sludges were kept in the inner part of 50 ml filter tubes with pores 0.45 µm in size (Maxi-Spin®, Alltech Inc., USA; see figure 6.1). The area within the Falcon tubes, outside of the filters, was also filled with salt, the lid was closed tightly and covered with a layer of Parafilm® (Pechiney Plastic Packaging, USA). A salt- toxicity control was set up

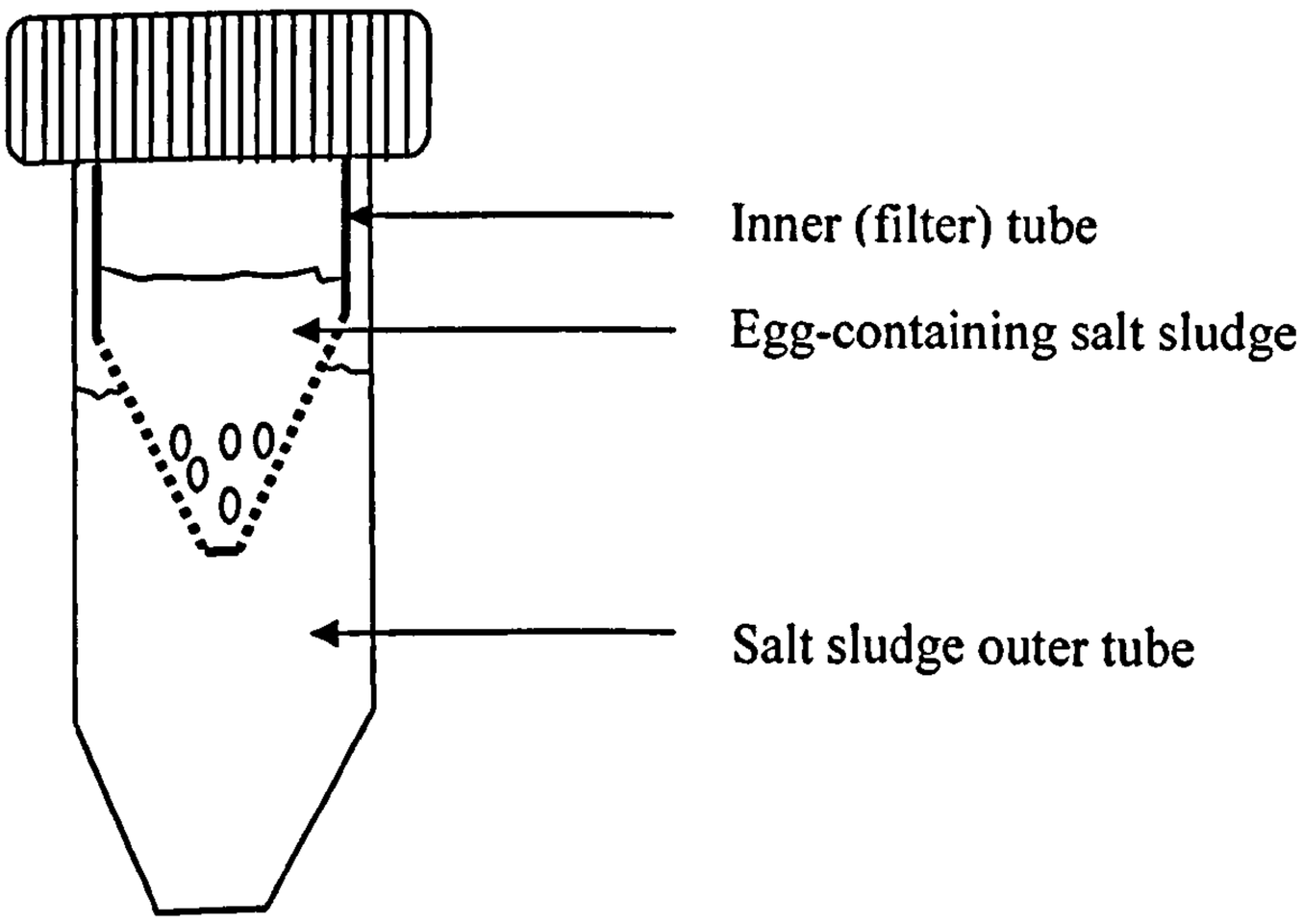


Figure 6.1: Schematic diagram of filter tubes.

for the 33% RH treatment as follows. Batches of chilled and non-chilled eggs were air dried at 20°C in a small concave glass dish after which this dish was put on top of the salt, within Falcon tubes. Care was taken to avoid any contact of the eggs with salt and the tubes were sealed in the same manner. As Parkin (1976) had shown that it was just the severity of the desiccation experience having an influence on the hatching process, and not the length of exposure to it, the effects of a single exposure time to salts was explored. After one week, during which the tubes were kept at 20°C, the inner, filter, part of the filter tubes was filled to the top with filtered water and the salts mixed with this water. The tubes were centrifuged at 2000 rpm until no water remained in the filters. This process was repeated 3-4 times, until no visible salt was present in the filter any more, and then another two times to wash any remaining salt off the eggs. Eggs were then transferred to a larger amount of filtered water and three replicas of approximately 500 eggs pipetted into wells as described in chapter 4. The eggs of the 33% RH salt-toxicity control were removed from the glass dish by pouring filtered water over the dishes and collecting them in a 500 ml glass bowl. The bowl was filled to the top and the suspension stirred. After the eggs had sedimented out the supernatant water was siphoned off and fresh filtered water added to the bowl, after which the eggs were pipetted into the wells. In all treatments, the number of eggs and larvae present were counted after 2 hours of returning the eggs to water, and the number of live and dead larvae counted on day 1 and then every other day until day 31.

Eggs that are severely damaged by either desiccation or salt toxicity may, as the result of rapid water influx and sudden egg expansion, rupture and release their larva on return to water. The number of hatched larvae present in wells on day nil, the day that eggs are put at

their hatching temperature, may therefore give an indication of the proportion of eggs damaged by the salt treatments. The influence of humidity treatment, and chilling, on the proportions of eggs hatching on day nil, within 2 hours of returning them to water, was tested in a two-way ANOVA. It was assumed that hatching induced by the influx of water, if this phenomenon existed, would commence rapidly after the return to water. The influence of chill and humidity treatment on the total proportions of eggs hatching on hatching day 1, and the proportions of eggs producing live larvae at this time, were analysed in a three-way ANOVA. This process was repeated for the total proportions hatching, which, for all treatments, had been completed on or before day 27. All analyses were carried out on arcsine-transformed proportions. The time taken to a fifty percent hatch of all treatments was analysed in a two-way ANOVA with factors 'chill experience' and 'humidity treatment'. In all analyses Tukey's pairwise comparison was applied for the humidity treatments. The proportions of eggs producing live larvae at the time of maximum hatching of the salt-toxicity controls were compared with their in-salt counterparts in a two-way ANOVA with factors 'salt contact' and 'chill experience'.

6.2.2 Larval migration onto herbage

All experiments were carried out in the laboratory at room temperature (20-24°C) and relative air humidity between 71 and 83% (as measured with a calibrated TinytagPlus® data logger; Gemini Data Loggers, UK).

Validation of larval recovery methods

The modified sucrose-interface larval recovery method, initially developed by Eysker and Kooyman (1993) and described in chapter 4, was used in all of the following experiments. The proportion of larvae recovered was measured in order to validate the method. Large numbers of pure culture *Teladorsagia circumcincta* and *Haemonchus contortus* L3 were obtained from the Moredun Research Institute. For both species separately, suspensions of approximately 2,000 , 10,000 and 50,000 L3 per 10ml of water were made by counting the number of larvae per 1 ml in a rostered nematode counting slide (Chalex Corporation, USA) and adding larvae or diluting with water, as appropriate. The number of larvae in 1ml of each suspension was now counted five times and the mean number, and 95% CI, computed. Three replicates of 10 mls of each suspension was carefully sprinkled onto approximately 100 grams of cut grass in empty large (40 litre) tubs, and the grass thoroughly mixed with a spatula and left to stand for 24 hours at room temperature. After 24 hours, water was added and larvae were recovered from herbage. Proportions of larvae recovered were calculated, arcsine transformed, and analysed in a two-way ANOVA with factors worm species and larval density.

Timing of maximum larval recovery

A suspension with mean larval density 3,333 *H. contortus* L3 per ml was created and, under continuous stirring, subdivided into 30 ml portions. These 30 ml quantities were, again under continuous stirring, subdivided into 10 ml portions. 4 plastic trays measuring approximately

1 x 0.5 m were filled with grass turf containing a variety of ryegrass (*Llolium*) spp. Grass length did not exceed 10 cm in any of the experiments and the attached layer of soil and grass roots was approximately 5 cm thick. The trays were subdivided into three replicate parts and 10 ml of suspension (containing approximately 33,333 L3) was, after parting of the grass, very carefully spread on the soil midline of the trays, using of a 20ml syringe and a blunted needle. The grass blades were thoroughly wetted with the fine mist of a plant spray after deposition of the larvae, and every 24 hours thereafter. After 24, 48, 72 and 96 hours, grass from each of the three replicates of one tray was cut with scissors at a height of 1-1.5 cm above soil level and immediately processed. Proportions of larvae recovered were arcsine transformed and analysed in a one-way ANOVA with factor time (days).

The influence of free water on vertical migration

Six more trays were set up and left to stand until the grass and soil layer were thoroughly dry. Two trays were seeded, as described above, with *T. circumcincta* larvae, two with *H. contortus* larvae, and two with 2933 *N. battus* L3 per replica. Trays were left for two hours after which the grass blades and upper soil layer of one tray per species was thoroughly wetted. After 24 hours the grass was cut and processed as above. The arcsine transformed proportions of larvae retrieved from wet and dry trays were compared in separate t-tests for each species. The Bonferroni-adjusted α-level was set at 0.017.

The influence of water on the emergence of larvae from dung

Dung containing pure culture *T. circumcincta* and *H. contortus* eggs was incubated at 25°C for 7 days. No water was added to the cultures during incubation. On day seven, 3 x 10 gram (g) of desiccated dung were crushed between two fingers and baermannised for 12 hours, and the numbers of live larvae calculated by counting those present in 3 x 1 ml of suspension, using a 1 ml nematode counting slide (Chalex Corporation, USA). Another 2 x 10 g were put in an oven at 90°C for 8 hours, in order to calculate the percentage dry matter and thence the number of larvae per gram of dry matter.

Four more grass turf trays were prepared as above (1 wet treatment and 1 dry treatment tray for each worm species) and 71 g of dung were spread evenly over each replica area. Wet treatment trays were thoroughly wetted as above, at the start of the experiment and every 24 hours thereafter. After 72 hours the grass was clipped from all trays, carefully avoiding picking up any dung particles with the grass, and processed immediately. Approximately 3 x 10 g of dung was picked up from each tray and the crushed pellets baermannised. Baermannisation of dry and wet dung was repeated on day 7 while the dry dung was also examined on days 14 and 21. On each occasion 2 x 10 g of each tray was also put in the oven as above, after which the number of larvae was corrected for dry matter content. Differences in arcsine transformed proportions of larvae recovered from the wet and dry treatments were analysed in a two-way ANOVA with factors treatment and worm species. Differences in numbers of larvae present on days 0, 3 and 7 were analysed in a one-way ANOVA for each tray, and a Bonferroni correction applied, setting the critical p-value at

0.013. Log transformed numbers of larvae present in dry dung were tested for significant decline with time using Pearson's correlation, with linear regression conducted if the correlation was significant. The slopes of the loglinear regression lines were compared and it was assumed that they were significantly different if their 95% confidence intervals did not overlap.

The fate of larvae initially not moving onto herbage

a. Vertical migration over time

The wet trays used to assess the influence of free water on vertical migration (see above) were, after initial clipping at 24 hours, kept at room temperature and sprayed daily with the same amount of water. On days 7 and 14 the newly grown grass was clipped and processed as above. Unfortunately, on day 21 limited grass re-growth prohibited clippings. It was assumed that the proportion of larvae recovered on herbage was a function of a) the number of larvae that had died since their application onto soil and b) the number of larvae removed in previous clippings of the same tray/replica area. The estimated proportion recovery on day t was computed as follows:

$$P(t) = \frac{\frac{1}{Mp} * Nr(t)}{N(0) - N\mu(t) - [\frac{1}{Mp}(Nr(t-6) + Nr(t-7) + Nr(t-13))]} \quad \text{Equation 6.1}$$

where Mp is the mean proportion recovery of larvae by the method used, as estimated above, Nr the absolute number of larvae recovered, $N_{(0)}$ the number of larvae applied on day zero and $N\mu$ the number of larvae predicted to have died, with $N_{\mu(t)} = \sum_{t=1}^{t=14} N_{(t-1)} * \mu$. The

estimation of the instantaneous larval death rate μ for *H. contortus* and *T. circumcincta* is described below. For *N. battus*, μ was estimated from the temperature experiments described in chapter 4 (see below). As the experiments were not run simultaneously for the three species and the cumulative effect of small differences in temperature experienced, RH, and amount of water applied over 14 days could not be estimated, inter-species comparison was not possible. The proportion of larvae recovered, corrected according to equation 6.1, were arcsine transformed and analysed in a one-way ANOVA for each tray.

On day 28 the trays were soaked in water and left to stand for 5 hours. The turf was rolled up and the water squeezed out into a bucket. The muddy water was passed over a 2 mm sieve and the mud remaining on the screen discarded. The process was repeated over a 1mm screen. The water was then passed over a 38μm screen and the material remaining on the screen divided over 2-4 Falcon tubes, depending on the amount of material. This fine sand was mixed with approximately 15ml of tap water. Larvae were then recovered from the sandy water in the same manner as described for the herbage plucks above. The remaining watery suspension of larvae was thoroughly mixed. 3 x 200 larvae were examined in a 1 ml nematode slide (Chalex Corporation, USA) and the number of live larvae recorded. The number of larvae applied to the *N. battus* tray proved insufficient to recover significant numbers of larvae in this way.

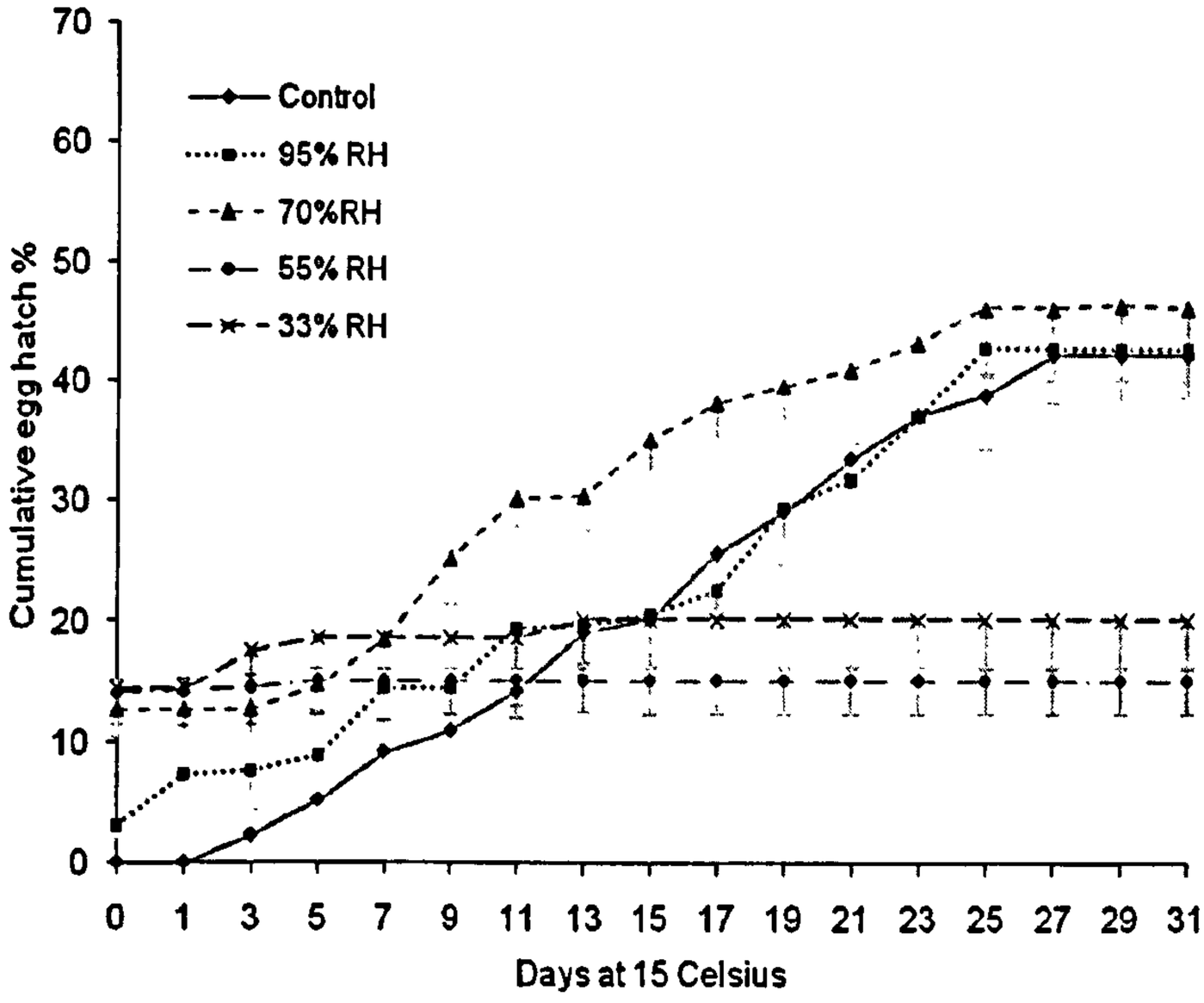
b. Survival in desiccated soil

On days 7, 14, 21 and 28 one quarter of the turf of the dry trays was removed, soaked in water and processed as above. 200 larvae were counted for each replica and the number of larvae alive counted as already described.

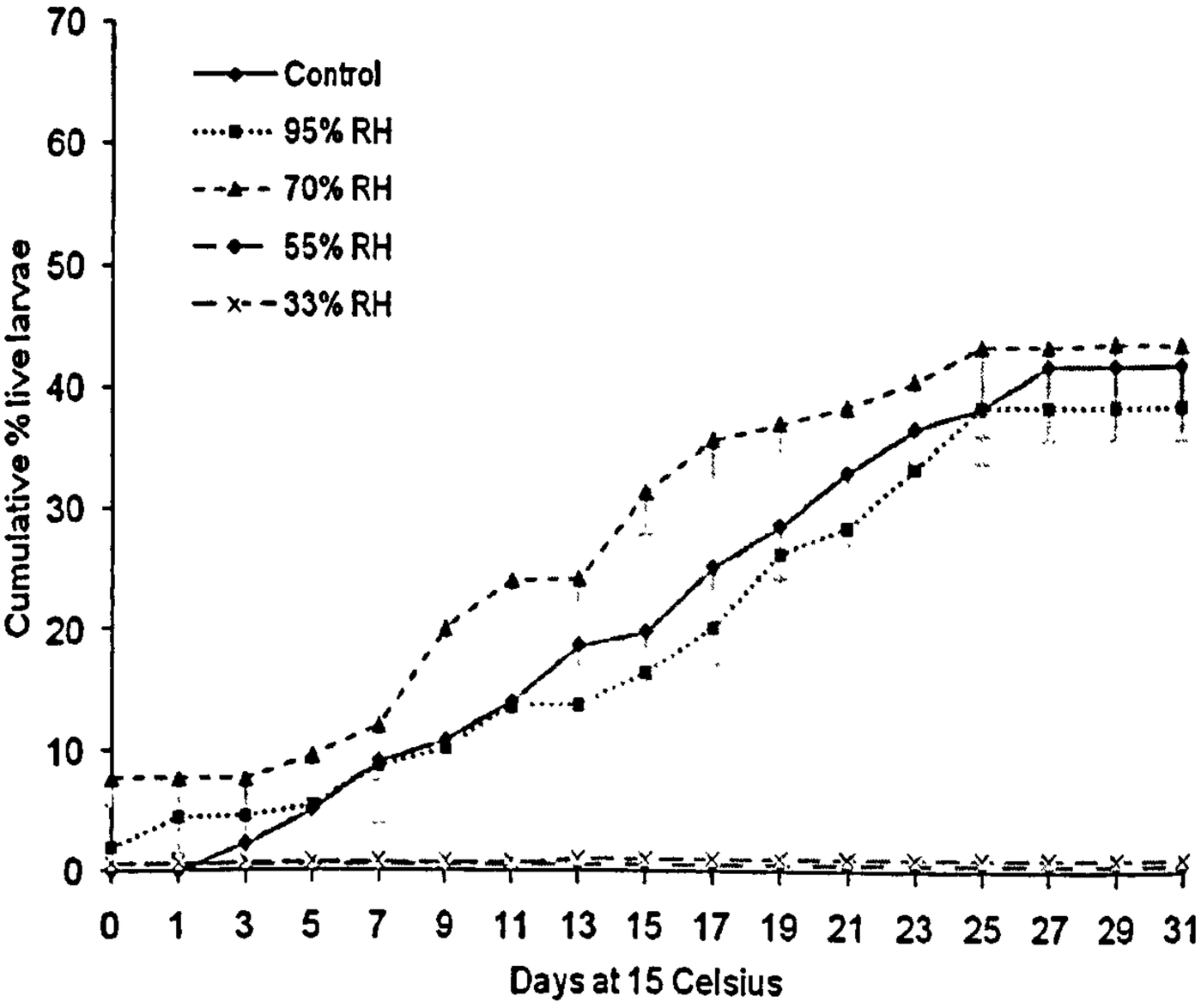
6.3 Results

6.3.1 The influence of re-hydration on the hatching process

The hatching of all eggs (figs. A), and of the eggs yielding live larvae (figs B), are illustrated in figures 6.2 (non-chill treatments) and 6.3 (chill treatments). In all RH treatments, a proportion of the eggs hatched on being returned to water. In the 95% and 70% RH treatments a small proportion of these yielded dead larvae, while in the 55% and 33% RH treatments virtually all larvae hatching on day nil were dead. None of these larvae showed signs of desiccation. All tightly filled their cuticle while showing the slightly arched, stretched out, position characteristic of larvae that have been dead for at least several hours, suggesting that they had either been dead for some time or were showing signs of over-hydration. The proportions of larvae hatching within hours of being returned to water was significantly higher in the 70, 55 and 33% RH treatments than in the other two treatments ($F_{4,29}$= 40.434, $p < 0.001$) but did not significantly differ from each other ($p \geq 0.608$). In the 95% RH treatment more eggs hatched than in the control ($p < 0.001$). Chilling had no influence on the number of eggs hatching on day nil ($F_{1,29}$= 1.067, $p = 0.314$).

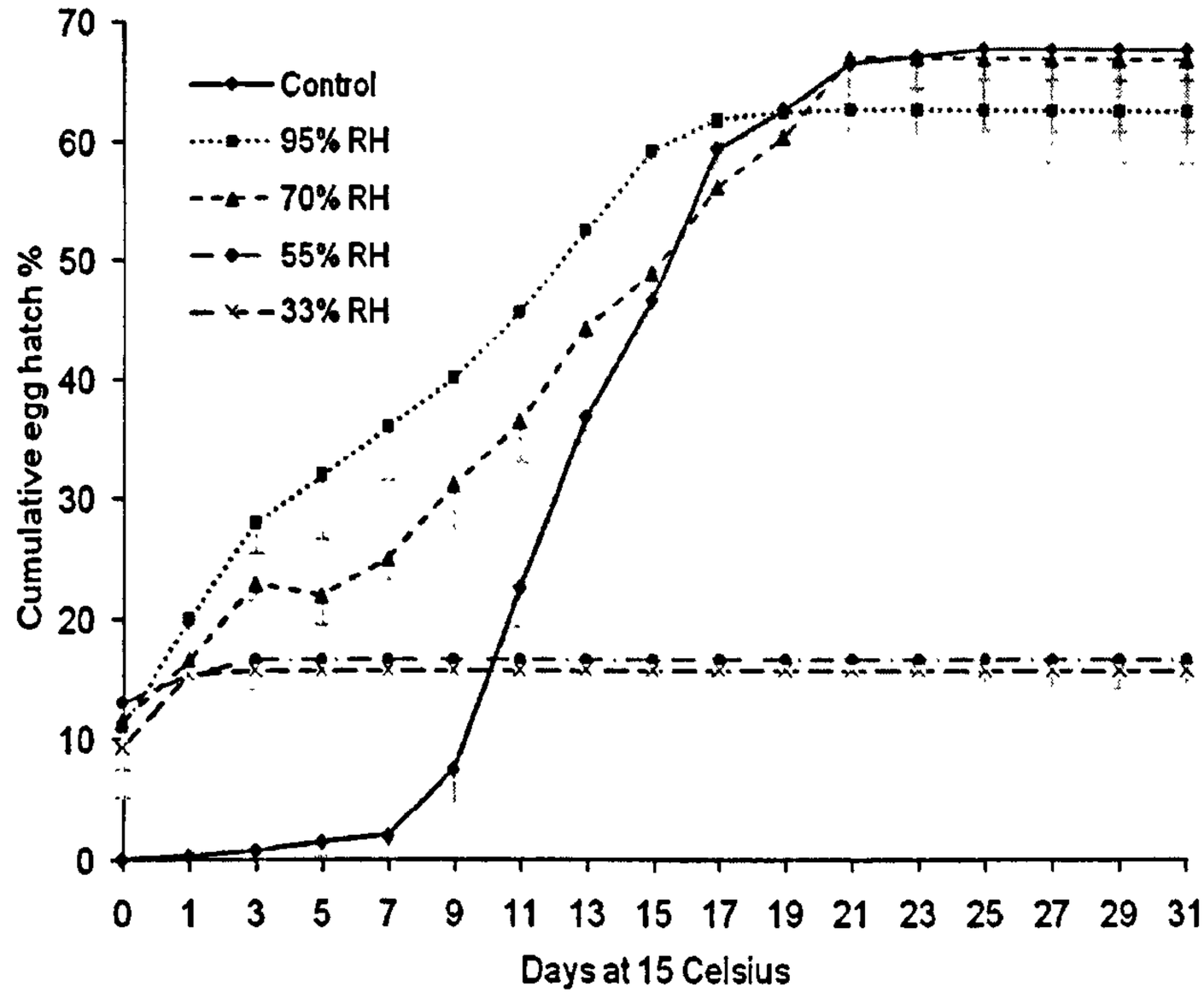


A. Cumulative proportion of non-chilled eggs hatching after re-hydration and placement at 15°C.

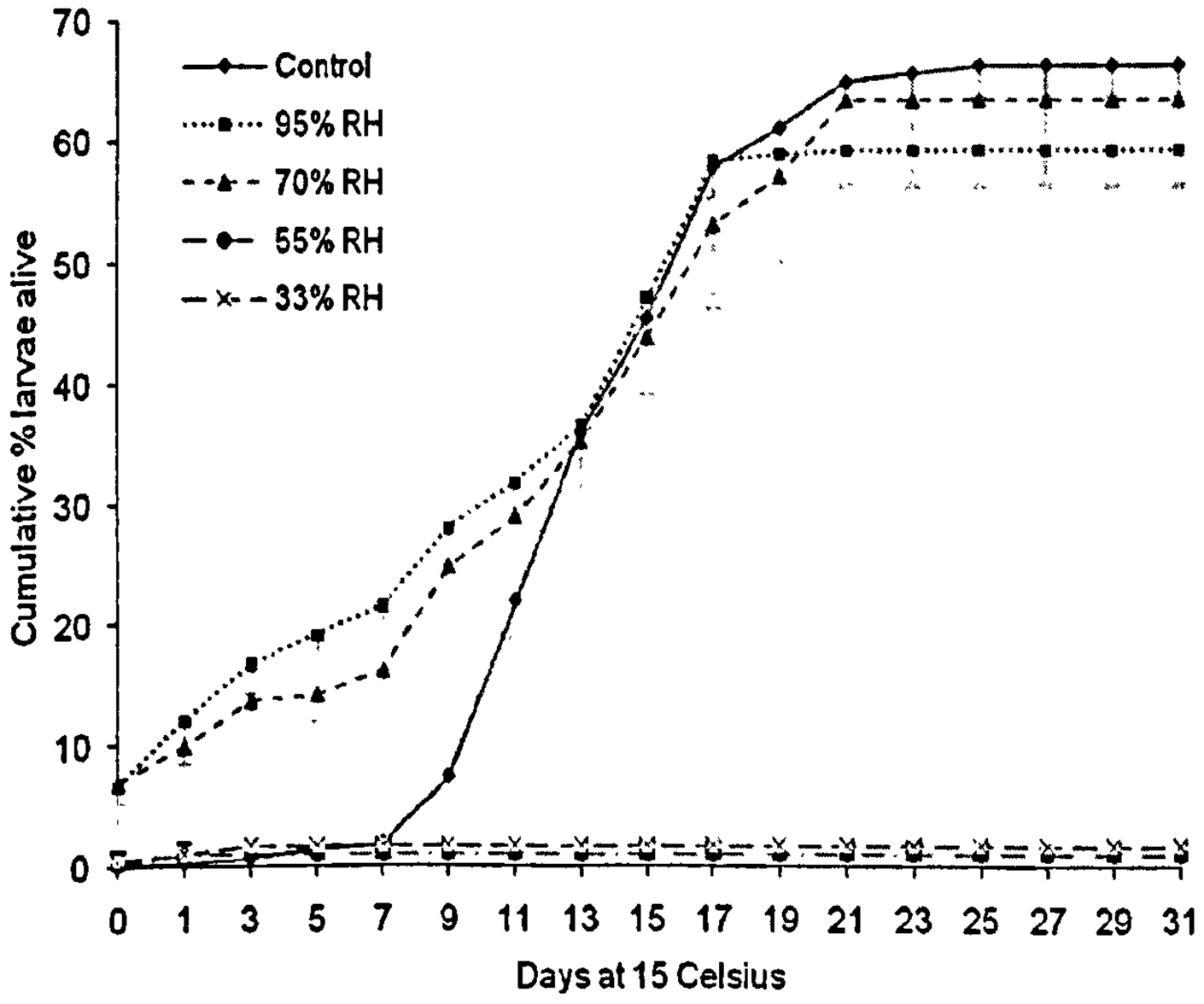


B. Cumulative proportion of non-chilled eggs producing live larvae; The 33% and 55% RH lines overlap.

Fig. 6.2 Proportion of *non-chilled* eggs hatching after their return to water. Error bars represent standard deviations.



A. Cumulative proportion of chilled eggs hatching after re-hydration and placement at 15°C.



B. Cumulative proportion of chilled eggs producing live larvae.

Fig. 6.3 Proportion of *chilled* eggs hatching after their return to water. Error bars represent standard deviations.

After day zero many live larvae hatched from the 95 and 70% RH treatments but hardly any from the 55 and 33% treatments. The 55 and 33% treatments were therefore omitted from any further analysis. On day one, more eggs had hatched in the 70 and 95% treatments than in the control ($F_{2,35}$ = 65,370, p < 0.001) while the 95 and 70% treatments could not be separated (p =0.516). Differences between the total proportion of eggs hatching and eggs yielding live larvae were significant ($F_{1,35}$= 30.868, p < 0.001) but significantly more live larvae were present in the lowered humidity treatments than in the control ($F_{2,35}$= 7.834, p = 0.002). In the chill treatments more eggs hatched than in the non-chill treatments ($F_{1,35}$= 36.370, p < 0.001) and dehydration significantly increased the proportion hatching ($F_{2,35}$= 8.547, p = 0.002). However, chilling had no influence on the proportions of eggs producing dead larvae ($F_{1,35}$= 0.766, p = 0.390).

At the time of *maximum hatching*, as expected, significantly more eggs hatched in the chill treatments than in the non-chill treatments ($F_{1,35}$ = 151.997, p <0.001) but the proportion hatching was not influenced by humidity treatment ($F_{2,35}$ = 1.962, p = 0.162). Significantly fewer eggs than those that hatched produced live larvae ($F_{1,35}$ = 5.843, p = 0.024) but there was no significant interaction with either humidity treatment ($F_{2,35}$ = 0.492 , p = 0.617) or chill treatment ($F_{2,35}$ = 1.322, p = 0.285). Again, the chilling of eggs had no influence on the proportion of eggs producing dead, or live, larvae ($F_{1,35}$ = 0.217, p = 0.646)

Regarding the *timing* of the hatch, even though differences between treatments spanned only a couple of days, dehydrated eggs reached the point of 50% hatch faster than eggs of the control treatment ($F_{2,17}$ = 21.700, p < 0.001). Chilled eggs reached the time taken to 50%

hatch significantly sooner than non-chilled eggs ($F_{1,17} = 40.000$, $p < 0.001$), and dehydrated, chilled, eggs hatched the earliest ($F_{2,17} = 34.300$, $p < 0.001$).

The 33% RH salt toxicity controls hatched in a manner very similar to those of the 33% RH in-salt treatments: both in the chilled and non-chilled treatments similar proportions hatched on day zero (mean 0.14 (0.098-0.15) and 0.16 (0.15- 0.18), respectively) and very few of these larvae were alive. In the following days a very small proportion of the eggs hatched. The proportion of eggs producing live larvae on the day of maximum hatch was significantly higher in the salt-toxicity controls ($F_{1,11} = 191.726$, $p < 0.001$) than in the in-salt 33% RH treatment. This proportion was also significantly higher in the non-chill salt control treatment than in the chill salt control treatment ($F_{1,11} = 72.487$, $p < 0.001$). However, the mean proportion of live larvae at the time of maximum hatch was only 0.07 (range 0.04-0.11), indicating that differences caused by salt toxicity were small.

6.3.2 Larval migration onto herbage

Validation of larval recovery methods

The proportions of *T. circumcincta* and *H. contortus* larvae recovered are given in Table 6.2.

The proportion of larvae recovered did not differ between species ($F_{1,17} = 1.883$, $p = 0.195$) or with the initial density of larvae ($F_{2,17} = 3.815$, $p = 0.052$). The mean recovery rate of larvae placed in the buckets was 0.092 (bootstrapped 95% CI 0.062-0.122).

No. larvae onto grass	2,000	10,000	50,000
T. circumcinta recovered	0.079 (0.063-0.092)	0.102 (0.080-0.117)	0.111 (0.091-0.125)
H. contortus recovered	0.079 (0.060-0.0101)	0.100 (0.089-0.110)	0.084 (0.077-0.092)

Table 6.2 Mean proportions of larvae recovered from herbage; Ranges are given between brackets.

Timing of maximum larval recovery

The percentages of *Haemonchus* L3 recovered after 24, 48, 72 and 96 hours are illustrated in figure 6.4. Percentages did not significantly increase or decrease after 24 hours ($F_{3,11}$ = 0.971, p = 0.489).

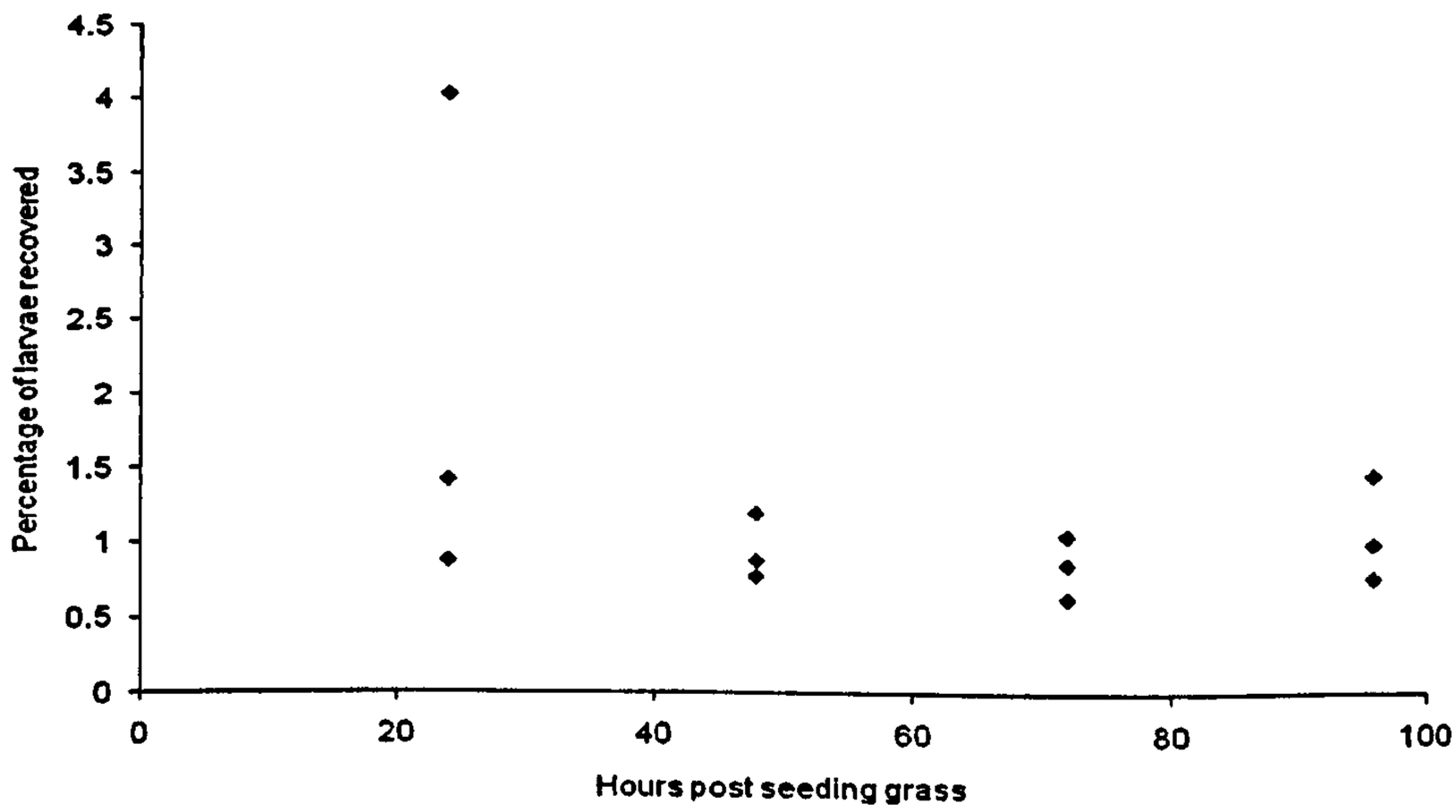


Fig 6.4 Percentages of *Haemonchus* larvae recovered at various hours after the seeding of grass turf with larvae; Each data point represents a replica.

The influence of free water on vertical migration

The percentages of larvae recovered from grass after 24 hours in the wet and dry treatments, and results of the t-tests, are given in table 6.3. Differences between wet and dry treatments were not significant for any of the individual species.

	H. contortus	*T. circumcincta*	*N. battus*
Wet treatment	0.45 (0.16-0.81)	0.89 (0.51-1.11)	0.24 (0.17-0.31)
Dry treatment	1.11 (0.75-1.44)	0.19 (0.12-0.30)	0.11 (0 – 0.23)
t statistic (df =2)	-2.41 (p = 0.095)	3.54 (p = 0.075)	1.29 (p = 0.323)

Table 6.3 Percentages of larvae recovered from the dry and wet treatments after 24 hours. The ranges are given between brackets. The *t* statistic is refers to dry and wet treatments of the individual species; The Bonferroni-adjusted α-level is 0.017.

The influence of water on the emergence from dung

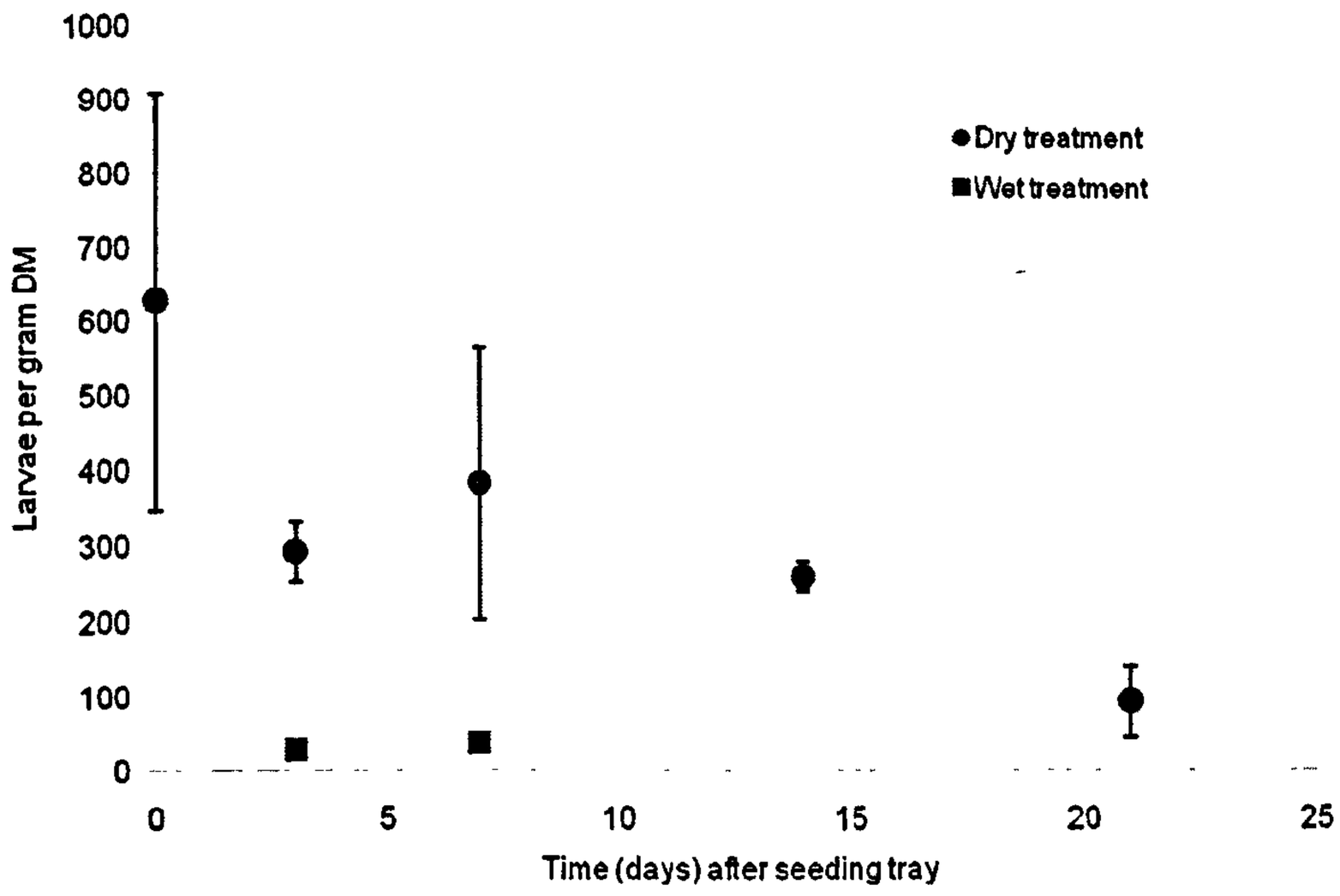
The percentages of herbage-recovered L3 from the dry and wetted dung trays are given in table 6.4. Significantly more larvae were recovered from the water-treated than from the dry trays ($F_{1,11} = 51.119$, $p < 0.001$). Significantly fewer *T. circumcincta* than *H. conctortus* larvae were recovered ($F_{1,11} = 35.315$, $p < 0.001$).

	H. contortus	*T. circumcincta*
Wet treatment	3.43 (2.96-3.94)	0.47 (0.40-0.55)
Dry treatment	0.18 (0.11-0.30)	0.04 (0.00-0.11)

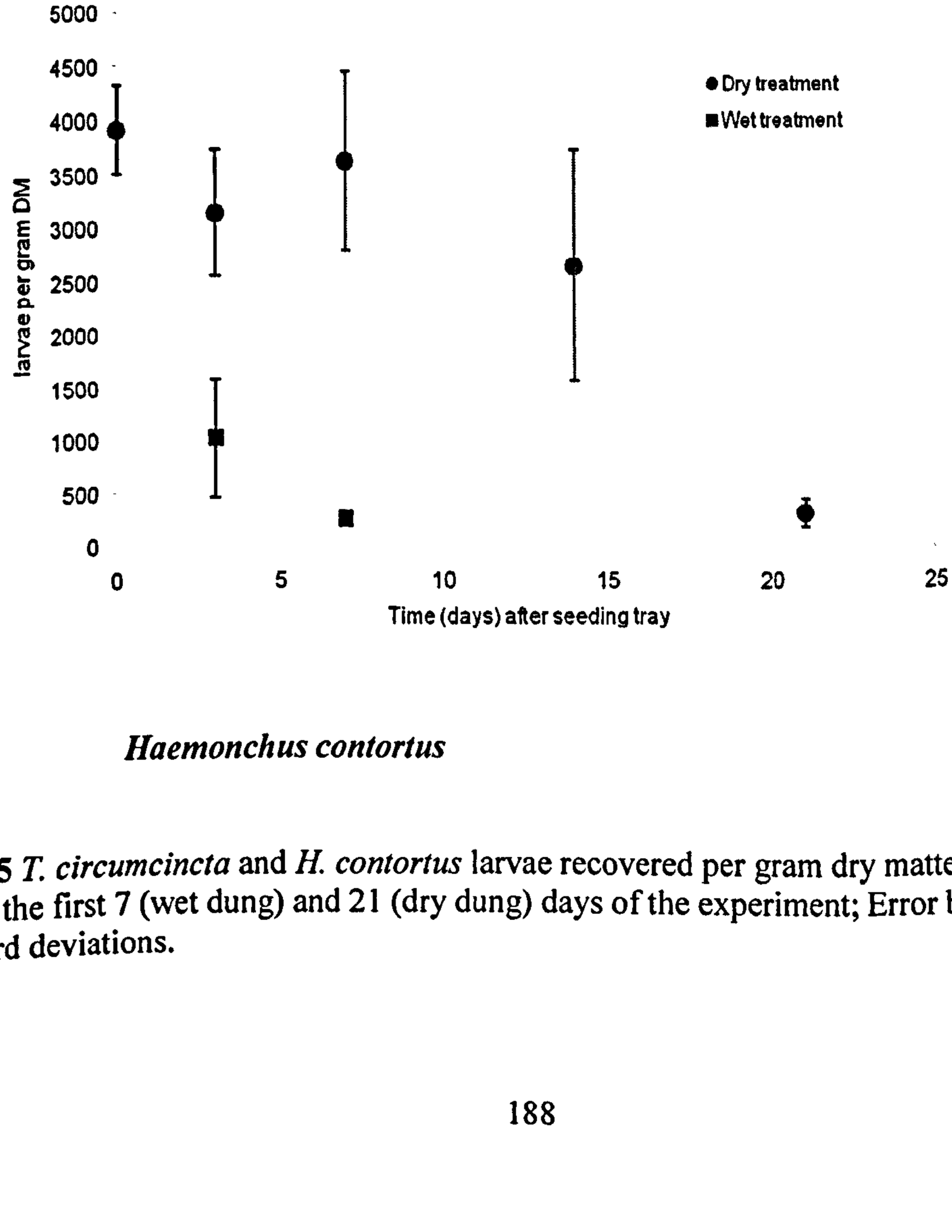
Table 6.4 Percentages of larvae recovered from the dry and wet dung treatments after 72 hours. Ranges are given between brackets.

The number of larvae present in one g of Dry Matter during 21 experiment days is illustrated in figure 6.5. In the wet treatments of both *T. circumcincta* and *H. contortus* the number of larvae recovered decreased significantly ($F_{2,8} = 13.526$, $p = 0.006$ and $F_{2,8} = 70.200$, $p < 0.001$, respectively). For both worm species the numbers recorded declined significantly between day 0 and day 3 ($p \leq 0.009$) but no further decline occurred between days 3 and 7 ($p \geq 0.120$). On days 3 and 7 numbers of larvae had not declined significantly in the dry treatments (*T. circumcincta*: $F_{2,8} = 2.146$, $p = 0.170$; *H. contortus*: $F_{2,8} = 1.612$, $p = 0.275$).

Over the 21 days, numbers of live larvae recovered from dry dung declined significantly for both *T. circumcincta* ($r_p = -0.824$, $p < 0.001$) and *H. contortus* ($r_p = -0.847$, $p < 0.001$). The slopes of the loglinear regression lines for survival in desiccated dung (*T. circ.*: 0.034, 95% CI +/- 0.007; *H. cont.*: 0.048, 95% CI +/- 0.008) did not differ significantly.



Teladorsagia circumcincta



Haemonchus contortus

Fig. 6.5 *T. circumcincta* and *H. contortus* larvae recovered per gram dry matter of dung during the first 7 (wet dung) and 21 (dry dung) days of the experiment; Error bars represent standard deviations.

The fate of larvae initially not moving onto herbage

a. Vertical migration over time

On day 28, a proportion of 0.60 (range 0.55-0.63) of *T. circumcincta* larvae present in the soil of the wet trays was found to be alive. A proportion of only 0.01 (0.00-0.05) of *Haemonchus* larvae had survived. Surprisingly, both values were very close to those predicted by the instantaneous daily dry tray soil survival rates (predicted 0.58 and 0.00, respectively; see under *b.*). Therefore dry-tray larval death rates were transferred to the wet trays. The instantaneous daily death rate of *N. battus* larvae was estimated to be 0.020 (Chapter 4). The corrected proportions, and the results of the ANOVAs are given in table 6.5.

Day	1	7	14	Test statistic
T. circumcincta	0.098 (0.056-0.122)	0.074 (0.024-0.136)	0.045 (0.015-0.068)	$F_{2,8} = 1.808$, p = 0.243
H. contortus	0.051 (0.020-0.091)	0.011 (0.00-0.020)	0.012 (0.00-0.018)	$F_{2,8} = 3.132$, p = 0.117
N. battus	0.072 (0.039-0.098)	0.023 (0.00-0.047)	0.070 (0.035-0.105)	$F_{2,8} = 2.553$, p = 0.158

Table 6.5 Mean proportions of larvae recovered, and their range, corrected for previously removed larvae and larval death.

Although, as described above, the numbers of larvae recovered from herbage do not increase over time, the soil represents a reservoir of larvae from which emergence on herbage may occur over time.

The corrected proportion of larvae recovered did not significantly increase or decrease over time for any of the three species. It appears that, at constant temperature and relative humidity, a constant proportion of live larvae is present on herbage.

b. Survival in desiccated soil

The proportion of larvae surviving in dry soil is illustrated in figure 6.6 Numbers of larvae recovered declined significantly over time for both *T. circumcincta* (r_p = -0.950, p < 0.001) and *H. contortus* (r_p = -0.926, p < 0.001). Mortality rates were significantly higher for *H. contortus* than for *T. circumcincta* larvae (Slopes of the loglinear regression lines *T. circ.*: 0.015, 95% CI +/- 0.002; *H. cont.*: 0.041, 95% CI +/- 0.011).

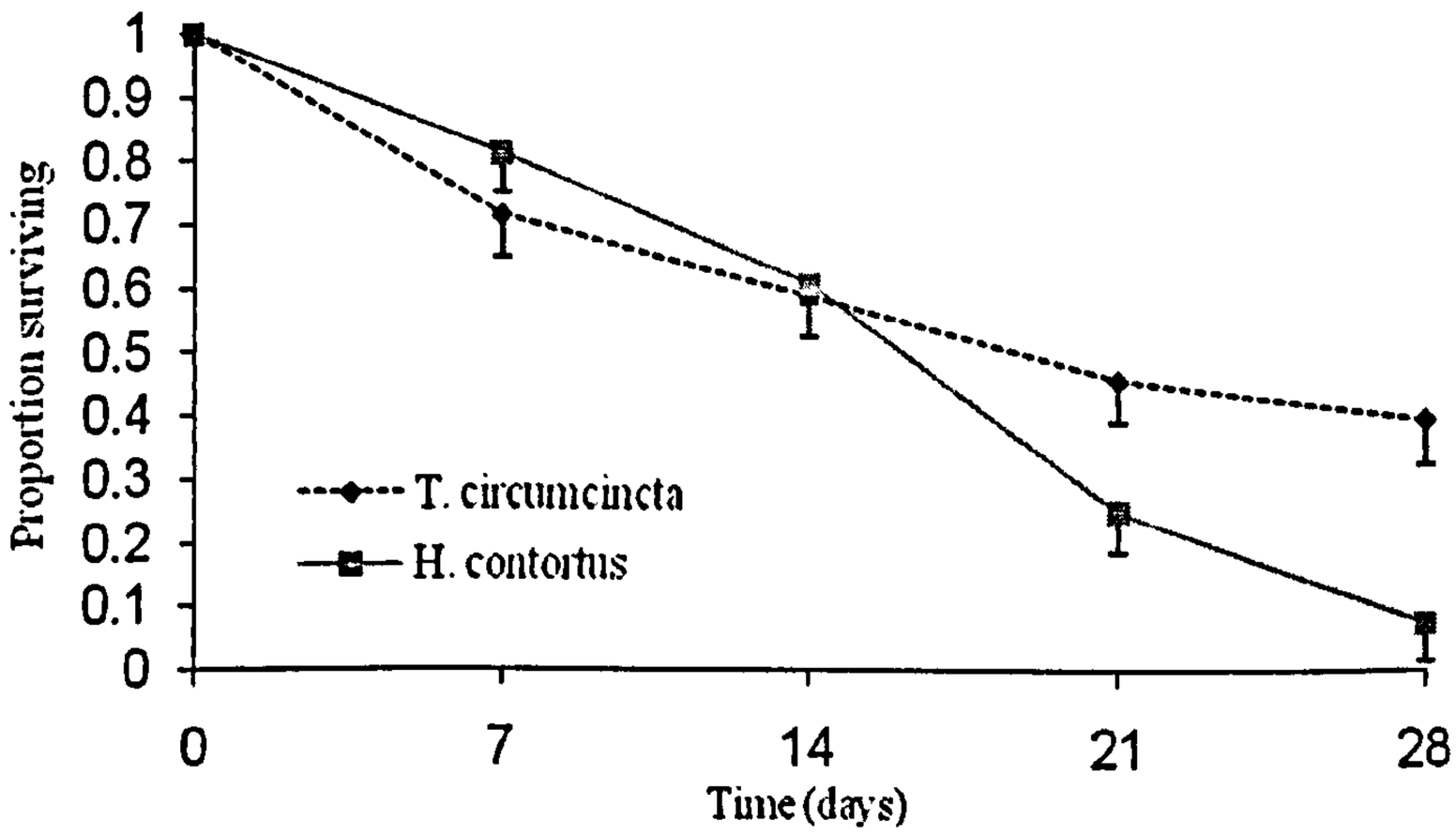


Fig. 6.6 The proportion of larvae recovered from desiccated soil alive at time (t); Error bars represent standard deviations.

6.4 Discussion

6.4.1 The influence of re-hydration on the hatching process

Within days of returning the eggs to water some significant hatching was recorded. A significant proportion of these eggs, as the result of the hygroscopic treatments, produced dead larvae. However, the number of live larvae present was, in the 95% and 70%, treatments, still higher than in the control. The salts producing relative humidities of 55% and 33% appeared to have damaged nearly all eggs irreversibly. Initially, a higher proportion of hatch was recorded in the lowered RH treatments but before or on day 12 of the hatching process, hatching of the controls had reached the same levels. From day 12 onwards the hatching pattern the 95% and 70% RH treatments was remarkably similar to that of the control. The more sudden start of the hatching process in the former two treatments did reduce the number of days taken for a 50% hatch of the population by a few days but at the time of maximum hatch proportions hatching could not be separated from the control while the proportion of eggs producing live larvae was no longer significantly influenced by humidity treatment. It appears that desiccation, followed by re-hydration, does not induce a mass hatch but may induce some mildly accelerated hatching.

The osmotic pressures applied in these experiments are likely to be higher than those experienced at pasture (Parkin, 1975b) and, at these lower osmotic pressures, desiccation of eggs may not occur at all. However, even if the osmotic pressures applied in these experiments are matched in the field the rehydration-induced hatching of approximately 10% of the population as live larvae is unlikely to account for the disease patterns observed in the

field, while increased death rates of eggs may also negatively influence parasite epidemiology when desiccation becomes too severe. Interestingly, chill treatment did not increase the proportion of salt-treated eggs releasing live larvae. On the contrary, in the chill treatments more eggs hatched on the first day after the return to water than in the non-chill treatments. There does not appear to be an easy explanation for this. From these results it appears that the hypothesis that trehalose protects the eggs of *N. battus* from desiccation has to be refuted. Waller (1971) compared the egg shell morphology of *Trichostrongylus colubriformis* with that of *Haemonchus contortus* and proposed that an inner layer of a lipid-like substance present in the eggs of the former, but not of the latter, was involved in its greater resistance to desiccation. Parkin (1972) detected a similar, amorphous, layer in the eggs of *N. battus* and reached similar conclusions. If such a layer indeed forms the main defence barrier against the loss of water it could explain why, in the present study, chilled and non-chilled eggs were affected in a similar way. Also, it could explain why the hatch of humidity treated eggs appeared to be a threshold phenomenon. At 95% RH treatment fewer eggs hatched on return to water than at 70%. Thus, at the hygroscopic pressures applied by the 70% RH treatment, more eggs were affected but not irreversibly damaged. However, in the 55% and 33% RH treatments virtually all eggs had died. This is consistent with a hypothesis of a layer preventing loss of water up to a certain threshold but not above. Also, on day nil highly similar proportions of eggs hatched in the 70, 55 and 33% RH treatments, suggesting that the egg shell may have been damaged in the same manner by all treatments.

The above also forms an argument against the involvement of salt toxicity in the hatching of eggs. It was decided to incubate eggs in, and not above, the salt solutions of increasing

hygroscopic strengths, the osmotic pressure of which quite likely exceeds those experienced by the eggs in the field. As a result, a lowered proportion of eggs releasing live larvae during the hatching process could be the result of a) severe desiccation of the egg, b) salt toxicity, or c) a combination of both. However, it is unlikely that the toxicity of three different salts would cause identical hatching effects on day nil. Dead larvae present on day nil did not have a distorted appearance but looked fatter than normal, suggesting that it was over-hydration that killed them, presumably because salt had leaked into the egg. Also, the 95% and 70% RH treatments produced the same proportion of live larvae as the control at the time of maximum hatch. The 33% salt toxicity control of the most severe humidity treatment showed a significantly higher proportion hatch than the in-salt treatments. Unfortunately, it cannot be estimated whether the lower proportion of live larvae produced in the in-salt treatments is due to salt toxicity or an increased osmotic pressure. However, it can be estimated that salt toxicity, in this treatment, contributed at most approximately 5% (range 2.1-9.9%) to larval death.

In conclusion, it appears that eggs exposed to high osmotic pressures at a RH of 55% or below die. A proportion of eggs exposed to lower osmotic pressures may hatch on rehydration but this proportion is very small compared to that of eggs hatching after exposure to temperatures within the hatching range. The extent of this type of hatching is unlikely to be of epidemiological importance and cannot account for disease observed after rainfall.

6.4.2 The influence of free water on the migration process

This work clearly shows that very few infective larvae are able to escape desiccated dung. The environment in which the developmental phase is completed (dung for most trichostrongyloids; soil for Nematodirae) is therefore likely to be responsible for important inter-species differences in pasture epidemiology.

For species such as *H. contortus* and *T. circumcincta*, if larvae develop during a hot, dry, summer week, a rain event has to take place before they are able to migrate onto herbage. Such a rain event would result in the mass release of larvae from dung and this may explain why clinical haemonchosis in sheep is often witnessed when rain follows a period of drought (VIDA data; personal, unpublished, observations at pasture). At times of the year when desiccation of dung is less severe, dew may suffice and larval release may be more gradual.

As, in contrast to the widely established opinion, it appears that parasitic nematodes do not need free water to climb onto herbage, *Nematodirus battus* migration is likely to take place whenever hatching occurs. The conclusion that free water is not needed for vertical migration, the result of the first study comparing the migratory behaviour of several species in a standardised laboratory setup, is supported by agreement in the results across the three species. A study comparing the influence of rainfall on the abundance of larval species at pasture, in Australia, reported a strong correlation between rainfall and the abundance of *T. circumcincta* and *T. vitrinus* and a striking difference with the presence of *Nematodirus* spp.,

which were not affected by rainfall (Niven *et al.* 2002). This finding is likely to reflect that water may indeed be limiting for migration out of dung, but not for migration onto herbage.

A reservoir function of sheep dung, with the potential for a sudden, dangerous, mass release of larvae, may play an increasingly important role under predicted climate change rainfall scenarios. Studies researching when pasture, previously grazed by infected animals, can safely be grazed again may have to include examinations of dung as well as herbage larval counts. The question now arises how long pasture dung can provide a depot of larvae for. An answer to this question is especially important for *Haemonchus contortus*, a species expressing fecundity rates approximately 10-20 times that of *T. circumcincta*. For both species, at a constant temperature approximating 20°C, the estimated instantaneous daily death rates in dung suggest that negligible numbers of larvae will be present after 3-4 weeks. At pasture, day-night fluctuations in temperature may increase, or decrease, these death rates. Chapter 3 found good agreement between temperature-predicted parasite abundance and disease incidence data, but predictions were made at a temporal scale of one month. In the UK, even in summer, the probability of a one-month drought is low. However, models aiming to predict larval peaks at pasture with more temporal precision may, depending on levels of dew, have to include rainfall data.

When 4 separate trays were cut on a single occasion, 1-4 days after larvae had been put onto them, maximum larval recovery from herbage occurred at 24 hours and no further accumulation of larvae on herbage occurred thereafter. Crofton (1948), Silangwa and Todd (1964) and Callinan and Westcott (1986) all reported maximum recovery rates 24 hours after

larval release. Also, regardless of recovery methods and species, larval recovery rates have been very consistently estimated at up to 2-3% (Crofton, 1948; Rees, 1950; Silangwa and Todd, 1964; Callinan and Westcott, 1986; Langrova *et al.* 2003) while they proved to be at similar levels and consistent between species in this study. It appears that larvae distribute themselves over soil and herbage within 24 hours. After the initial 24 hours a constant recovery rate of larvae from herbage over time was witnessed. The most likely explanation for this is continuing random movement.

Fenton and Rands (2004) developed a model framework describing trade-offs between resource depletion of the infective stages of parasites and host encounter rate and showed that the optimal migration strategy strongly depends on the probability of host contact. It was proposed that the infective stages should, depending on the benefits of increasing the probability of host encounter and the attached costs, either adopt a sit-and-wait strategy or actively 'move towards' a host (a 'cruising' strategy). The latter strategy may be changed to a sit-and-wait strategy when energy reserves fall below a certain threshold level. Fenton and Rands (2004) gave gastrointestinal nematodes as an example of parasites expressing this switch: once on the grass blades, further cruising would not be advantageous and thus a sit-and wait strategy would be adopted.

Once out of dung, as their hosts are unlikely to graze close to faecal deposits unless forced to do so by grass shortage (Hutchings *et al.* 2006), the probability of ingestion of L3 is likely to increase with the distance travelled. However, as energy reserves are finite, further migration is traded off with decreased survival on herbage. Skinner and Todd (1980), working with

Haemonchus contortus, found that, although some larvae were able to migrate as far as 90 cm in 24 hours, over 90% were found within 10cm of the faeces. Williams and Bilkovich (1973) and Langrova et al. (2003) published very similar results for the lateral migration of *Ostertagia ostertagi* and cyathostominae, gastrointestinal nematode affecting cattle and horses, respectively. These findings, which, once more, highlight the importance of regular moves of farmed animals to 'clean' pasture, seem to support a cruise-then-sit-and-wait strategy. As it is unlikely that free-roaming ruminants would graze within such distances of faecal deposits they may suggest that the migration behaviour of larvae is influenced by the domestication of their hosts and modern farm practices. The distance travelled may also correlate with the probability of rapid disintegration of dung in a certain climatic region, and rainfall may therefore indirectly influence larval migration.

As larvae climb grass blades they are increasingly exposed to lowered relative humidites and other noxes, such as sunlight (Tromba, 1978). Thus, 'cruising' is not only beneficial but also harmful and this is likely to present a second trade-off similar to the first. The consistent finding of 90% of recovered larvae being present within the lower 2.5 cm of the grass stems (Crofton, 1948; Rees, 1950; Silangwa and Todd, 1964; Callinan and Westcott, 1986), the minimum height at which, depending on host species, ingestion may occur is likely to be the result of this trade-off. If larvae would just adopt a sit-and- wait strategy once on grass, then larval numbers on grass would increase over time until the increased death rates would be greater than the rate of migration onto herbage. Under the laboratory conditions presented the latter is unlikely to be the case. The present findings suggest that larvae remain in a cruising mode. They support the hypothesis by Crofton (1948) of continuous, random,

movement of larvae within certain temperature and humidity thresholds. Grenfell *et al.* (1986), modelling migration and mortality of *Ostertagia ostertagi* and *Cooperia oncophora*, even showed that models in which the migration rate was held constant showed as good an agreement between observed and predicted larval counts as models in which the rate was a function of microclimate. Obviously, whether this truly reflects reality or not depends on the time-steps used in the model and, quite likely, the season of the year. Crofton (1948) measured the RH, temperature and light intensities at different heights along herbage stems. He was able to relate these measurements to the height at which the larvae of *Trichostrongylus retortaeformis* were found on different types of herbage. He proposed that larvae moved at random as long as RH and temperature were favourable, while halting or re-directing their movement when travelling along a higher to lower RH gradient. Yamada and Oshima (2003), studying cues for the migration behavior of *Caenorhabditis elegans*, described how this species actively avoids higher temperatures, moving along a higher to lower temperature gradient. Rees (1950) measured *H. contortus* larval recovery on plots at 2-hourly intervals. She found that, as long as temperature was not limiting for the movement of larvae, the numbers of larvae recovered from herbage differed very substantially even between 2-hour measurements and were a function of relative humidity; In other words, larvae re-distributed over the grass stems during the day as the result of changes in micro-climatic circumstances. It appears that the migration of trichostrongylid larvae is adequately modeled by either a continuous cruising strategy or a cruise, sit-and-wait, cruise strategy. Although the distance vertically climbed appears to be influenced by a trade-off between the probability of ingestion by a host and increased death rates higher on the stem, movement from soil onto herbage stems seems to occur at random. Thus, the first few centimetres of

soil, and the mat of dead vegetation on it, remain reservoirs for larval emergence onto herbage and consistent percentages of the larval population will appear on herbage until all have died. Larval migration rates, estimated over the first 14 days, were, again, shown to be consistent between species. The presented work suggests that, even during dry summer weeks, live larvae may remain present in the soil for up to one (*H. contortus*) or two (*T. circumcincta*) months. At the constant temperatures of the laboratory set-up, as shown by the longitudinal study, even in moistened soil the number of larvae present on herbage may fall below detection level before then. However, at pasture, a longer period of 3 months has been suggested (Eysker *et al.* 2005a). The longer soil survival at pasture may be the result of decreased larval activity during the night as the result of cyclic temperatures.

6.5 Conclusions

Available methods for the recovery of larvae from herbage are sensitive enough to investigate and quantify migration behaviour. The findings were very consistent between the larval species, and also with the existing literature, indicating that results of migration studies are widely transferable between trichostrongyloid species.

In the temperate regions, moisture availability is unlikely to limit egg development.The well documented positive correlation between rainfall and the emergence of larvae on herbage, with subsequent increased disease incidence, is most likely the result of the release of larvae from a dung reservoir. Sudden increased availability of water did not appear to induce mass hatching of *N. battus* eggs. Moreover, water is not a limiting factor for the migration of

larvae which have escaped dung. Therefore, the emergence of *N. battus* larvae, at the time of hatching already incorporated in the soil, after a period of rain is more likely to be temperature related: on cloudy days a lowered maximum temperature may result in hatching opportunities.

The migration behaviour of trichostrongyloids appears to be the result of two trade-offs. When travelling in a horizontal plain, larvae are protected from the elements by soil and herbage. The distance travelled is determined by a trade-off between energy reserves and the probability of ingestion by host. In a vertical plane, larvae travel only a short distance, the length of which is influenced by exposure to damaging environmental influences and the probability of ingestion. As the continuous larval movement (cruising) is spatially *at random*, the proportion of a larval population present on herbage is very constant in time and declines with the rate of larval death of larvae in the soil reservoir. The height at which larvae are found on herbage varies with microclimatic conditions.

In terms of working towards a model of *Nematodirus battus* epidemiology it can be concluded that rainfall may only have to be included to model potential egg losses and prolonged egg development phases as the result of delays in incorporation of desiccated dung into the soil. In the next chapter it will be attempted to quantify these delays.

Chapter 7 – Modelling the epidemiology of *Nematodirus battus*

This chapter consists of two parts. Part one explores ways in which modelling of the ecology of *N. battus* can be simplified and makes use of stripped-down models of egg development and hatching. In particular it focuses on the modelling of time delays between the completion of egg development and hatching, and the probability of completing the hatching process. Part two first attempts to estimate the contributions of autumn infections to total on-farm egg output in a longitudinal study of worm egg counts in lambs. It then connects all leads in a simple, stochastic, full-cycle model of *N.battus* epidemiology.

7.1 Modelling the ecology of the free-living stages of *Nematodirus battus*

7.1.1 Introduction

The need for a model

The population dynamics of *Ostertagia/Teladorsagia* species (e.g. Grenfell *et al.* 1987a; Smith and Grenfell, 1994) and, to a lesser extent, *Haemonchus contortus* (Smith, 1988; Smith,1990), have been extensively modelled whereas currently no such models exist for *N. battus*. Ollerenshaw and Smith (1966) and Smith and Thomas (1972) developed simple linear regression models, modelling the onset of the spring peak as a function of one-foot soil temperatures taken in March, in an attempt to forecast the onset of a period of risk to young lambs. Although, in some years, the hatch occurred two weeks earlier or later than predicted, as their model basically predicted a hatch when the soil temperature

is rising their predictions were, on the whole, fairly accurate. However, such models take into account a lower threshold for hatching only while the hatching dynamics of *N. battus*, under conditions of global warming, are likely to be increasingly under pressure from the upper threshold (chapter 4). A model describing not only the timing but also the shape of the peak is needed, as it is the shape of this peak which, assuming hosts are present, will ultimately determine the risk of disease to the host.

Egg cohorts and their development

The hatching patterns of *N. battus* pose new challenges to the modelling of the pool of embryonated eggs available for a spring or autumn hatching opportunity. The eggs of trichostrongyloids normally readily hatch when the L1-stage larvae has developed. Therefore the development of free L3, ready to migrate onto herbage from dung, can be modelled by a temperature dependent development rate, and newly developed L3 added to the pool of infectious larvae present at pasture. For *N. battus* eggs, however, the events of the completion of development and hatching are time lagged. These time lags vary for each cohort of eggs completing development while, as the developmental stage of *N. battus* is relatively long, the time taken for the completion of development may also vary very considerably between cohorts. This makes it very challenging to program a model of the fate of cohorts of daily or weekly outputs of eggs. Moreover, the chilling experience, and thus the percentage of eggs of the cohort hatching at a given hatching opportunity, would have to be modelled separately for each cohort, complicating matters further. Therefore, the question arises how the number of cohorts can be reduced and whether there is a moment in time where virtually all eggs have the same level of chilling experience.

From the work by Thomas and Stevens (1960) and Gibson and Everett (1981) it appears highly likely that eggs produced during the spring peak will invariably be fully embryonated before the start of the autumn hatching opportunity. However, as the work was carried out in southern parts of the UK, it is not clear whether this is also the case in Scotland, where the spring peak will be later and the autumn peak may be earlier than in the South. Also, eggs are not exclusively excreted in spring or autumn. Although a phase of worm expulsion normally follows the high levels of infections taking place during peaks of larval emergence (Lumley and Lee, 1981; Winter *et al.* 1996), more than 40% of animals do not eliminate the total worm burden after the initial infection (Taylor and Thomas, 1986; Israf *et al.* 1996) but reduce it only up to a certain threshold level (Conwill Jenkins and Phillipson, 1970; Medley, 2002). Depending on the time of excretion, eggs excreted by these animals may or may not be embryonated in time for the autumn peak.

Animals infected during the autumn peak begin to shed eggs approximately 20 days (Mapes and Coop, 1972) after the start of the autumn hatch. By this time the mean temperature is likely to have already fallen below the development threshold, which approximates to the lower hatching threshold (chapter 4). Similarly, in the following spring, development and hatching start at approximately the same time. It is therefore highly unlikely that autumn-produced eggs will contribute to the following spring peak.

It appears that, with respect to the modelling of cohorts of eggs deposited in the same year, there is one important cut-off point and this is the day at which eggs deposited during the summer will not be able to complete their development before the start of the autumn peak. The timing of this cut-off point depends on the timing of the autumn peak. Also, at this point in time, none of the eggs in the cohort have any chilling experience, a

situation again simplifying the modelling of the one, large cohort. Both Gibson and Everett (1981) and Thomas and Stevens (1960) found that eggs put out at pasture before or during August were able to contribute to the following Spring peak whereas eggs put out later were not. This suggests that such a cut-off date may indeed exist and be timed in August. Eggs deposited after this cut-off date would experience their first hatching opportunity in the autumn of the next year.

In chapter 6 it was proposed that the degradation of dung, which is likely to be dependant on rainfall, may be an important factor in the timing of the onset of development. This chapter will assess, on average, how much time the degradation of dung adds to the development period and whether spatial differences in rainfall patterns are likely to lead to differences in developmental time.

The aims of the development model are to explore

(i) how the modelling of the developmental phase of *N. battus* can be simplified

(ii) whether the influence of spatial climatic differences on egg development can account for differences in parasite epidemiology described in chapter 2

(iii) ways in which climate change is likely to alter the pools of eggs available for spring and autumn hatches.

Hatching patterns and disease risk

Apart from the timing of the presence of susceptible hosts, which in itself is determined by the timing of lambing and pasture rotation strategies, the likelihood of clinical disease in a flock is influenced by at least three factors: the number and concentration of days

with minimum temperatures above and maximum temperatures below the hatching thresholds ('hatching days') in spring, the number of eggs deposited in the previous year and the number of eggs that failed to hatch in the previous year. It is currently not known which of these determines the between-year differences in the level of clinical disease experienced (see chapter 2), and whether all three factors are determinants at all.

In chapter 2 it was identified that the disease risk is higher in Scotland than in the Southwest of England. An investigation into the reason behind this may supply us with an important insight into the essence of *N. battus* epidemiology. A number of hypotheses can be formulated:

1. A significantly later Spring peak in Scotland results in somewhat older lambs being present at pasture and these will consume larger amounts of herbage during the hatch;
2. The Scottish Spring climate provides the parasite with more hatching days in a shorter period of time, leading to 'spikier' peaks;
3. The number of eggs contributing to the Spring peak is larger in Scotland as the result of either differences in the timing of egg deposition or in the degree to which non-chilled larvae hatch;
4. In Scotland, as the result of climate change, the proportion of spring-deposited eggs reaching the L3 stage before the autumn hatching window has increased;
5. Speedier incorporation of eggs in the soil leads to smaller losses of undeveloped eggs in dung in Scotland;
6. In Scotland, fewer eggs are killed during the summer as the result of heat stress;

An important first step towards testing these hypotheses is to test whether climatic conditions for hatching are likely to differ significantly between regions. Most of these

hypotheses can then be tested in a simple model of parasite ecology while others will be discussed in part two of this chapter.

The model aims to:

(i) quantify disease risk to the host;

(ii) explore how climate, and future climate change, is likely to influence the timing and shape of peaks of larval emergence;

(iii) investigate whether, for this parasite, climate is a likely driver for a bet-hedging approach to hatching;

(iv) consider possible reasons for the surge in cases of clinical Nematodiriosis in Great Britain, in Scotland but not further south;

7.1.2 Model construction

Conceptual framework

The model only wants to investigate the processes influencing the magnitude and shape of peaks, the readiness of eggs for hatching and hatching conditions, and differences in these processes between UK regions, without describing actual numbers of eggs hatching from one year to the next. The fate of eggs and larvae is simply followed through time, without any assumptions for host presence and grazing management. Figure 7.1 gives an overview of the important life-cycle stages of the free-living stages of *N.battus* and the state variables and parameters are defined in tables 7.1 and 7.2, respectively. Eggs are deposited in dung and incorporated in the soil where development to the L3 stage takes place. After development is completed hatching takes place only after a temperature

dependent stimulus. A certain proportion of larvae hatches at the fist encounter of this stimulus while the remainder remains in the egg until a chilling process is completed. Free L3 will then only be able to infect hosts after migration onto herbage is completed.

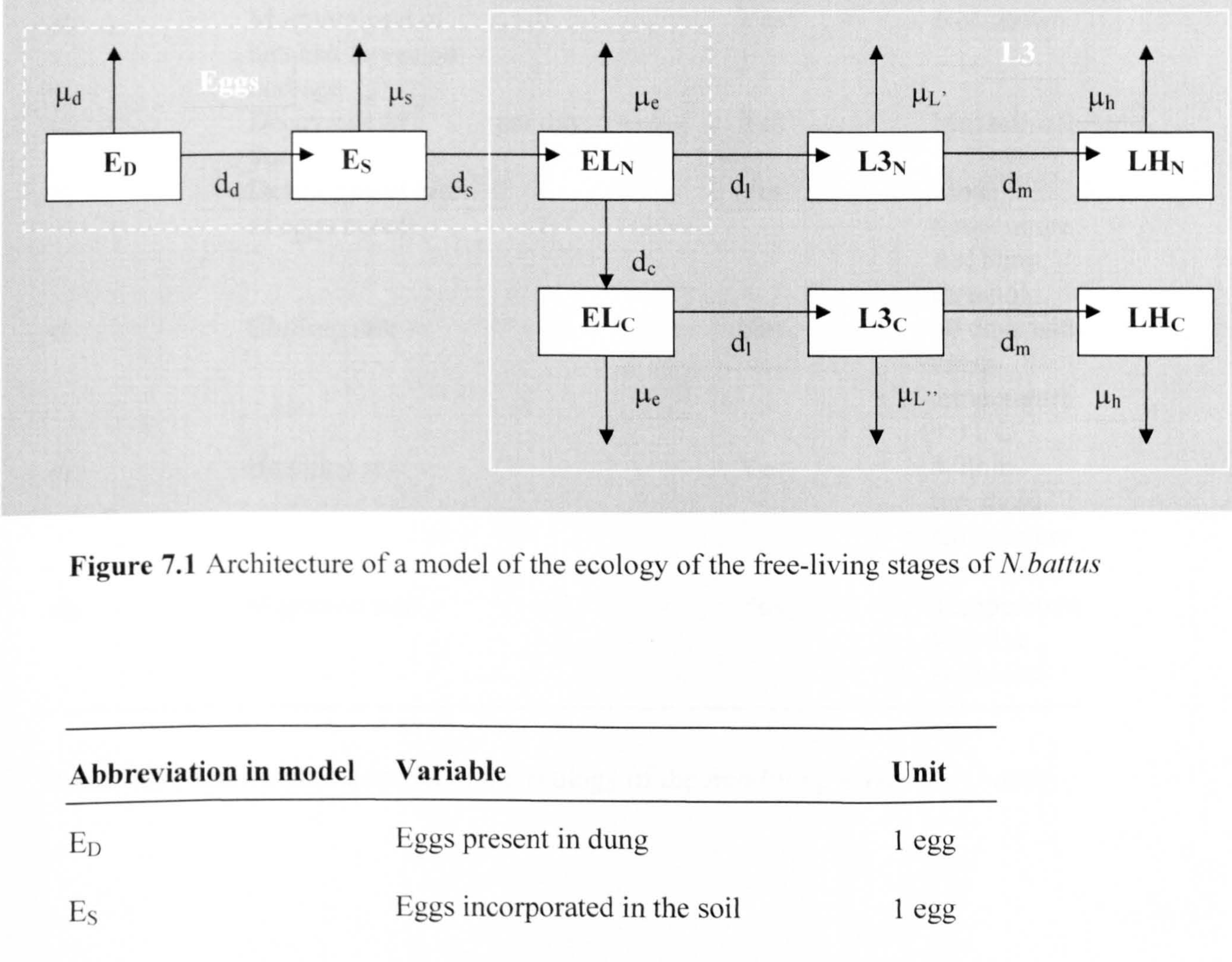


Figure 7.1 Architecture of a model of the ecology of the free-living stages of *N.battus*

Abbreviation in model	Variable	Unit
E_D	Eggs present in dung	1 egg
E_S	Eggs incorporated in the soil	1 egg
EL_N, EL_C	Non-chilled (N) and chilled (C) embryonated eggs	1 egg
$L3_N$, $L3_C$	Non-chilled and chilled L3 present on or in the soil, respectively	1 larva
LH_N, LH_C	Non-chilled and chilled L3 on herbage	1 larva

Table 7.1 State variables of a model of the ecology of the free-living stages of *N.battus*

Abbreviation	Parameter	Unit	Constant	Depends on
μ_d	Egg mortality rate in dung	Instantaneous rate/ parasite/day	Not known	Not known
μ_s, μ_e	Mortality rate of unembryonated and embryonated eggs	„	No	Temperature (> 25˚C)
μ_L, μ_L''	Mortality rate of hatched larvae in soil	„	No	Temperature
μ_h	Mortality rate of hatched larvae on herbage	„	Yes	Not known
d_d	Decay rate of dung	per day	Yes	Mm rainfall
d_s	Development rate of eggs in soil	''	Yes	Mean temperature and temp. threshold
d_c	Chilling rate	''	Yes	30 days with mean temperature < 11˚C
d_l	Hatching rate	''	Yes	Within-threshold temperature experience
d_m	Migration rate	''	Yes	Temperature, Relative Humidity

Table 7.2 Parameters of a model of the ecology of the free-living stages of *N.battus*

The dynamics of such a model are very hard to understand, and the output of larval peaks difficult to interpret, without understanding the behaviour of the building blocks.

Therefore it was decided to break it up in a development and a hatching model, and to strip the two models of complexity at the start, adding it again if necessary (van Nes and Scheffer, 2005).

Parameters

Egg development

The value of μ_d is not known. However, when comparing regions, this parameter starts to matter only if differences in soil incorporation rates are found between regions. Therefore it was decided to not to investigate and to re-visit on indication of the model. From chapter 4 the death rate of unembryonated eggs at constant 30˚C was approximated as 0.006/day while 4-week storage of embryonated eggs at 25˚C did not reduce hatching rates. The transformed (see Appendix) 30-year daily mean temperatures of the weather stations Yeovilton (Southwest), Waddington (Midlands) and Paisley (Scotland) were studied for temperatures ≥25˚C. Over the past 30 years, temperatures in this range occurred most frequently in the Southwest but, on average, only on 0.2 days per year (with a maximum of 1 day). If all measured temperatures were increased by 2˚C mean temperatures ≥25˚C still only occurred 1.1 days per year (maximum 6 days). Therefore it was decided not to include temperature-dependent egg death in the model and to model egg development stages only (as summarised in figure 7.2, page 214). It may be inappropriate to model egg death on mean temperatures but rates of maximum temperature-based egg death are not available.

The development model was run on 30-year transformed mean daily temperature data, and rainfall data, from the three weather stations as described in chapter 3. The model was run forward in time from the predicted spring peak and backwards from the predicted autumn peak. In order to seed the model with eggs at the appropriate time, and to determine the cut-off point, additional parameters were recorded as follows.

(i) In order to describe the development of spring eggs the following were determined:

• *Peak hatch day*

If, after prolonged periods of weather conditions adverse for hatching, the temperature remains within the hatching thresholds hatching will start from day 8 onwards and be completed over the following 8 days (chapter 4). For each year the peak hatching day was therefore determined as the 16th day of the year for which the minimum and maximum temperatures stayed within hatching threshold (11-17°C). If no 16 hatching days occurred before the start of July then the maximum number of hatching days up to this point was taken.

• *Peak egg output*

Spring egg output was summarised as one day, being 20 days after the peak hatching day (i.e. just before the mass expulsion of adult worms occurs; Lumley and Lee, 1981).

• *Faecal matter decay rate (d_d)*

The decay of faecal matter may be influenced by rainfall and by temperature (Levine and Anderson, 1973). The temperature influence is likely to be related to the activity of insects, and other invertebrates, incorporating faecal matter into the soil (Holter, 1979). As, in this model, the development of eggs deposited from the spring peak to the autumn peak of the same year was studied, it was assumed that the temperatures, at these times of the year, were above the threshold for activity of these animals. From the data presented by Niezen *et al.* (2003), who measured faecal decay at pasture in New Zealand, daily faecal decay can be approximated as a linear processes running at rates of 1/30 per day in spring and summer and in autumn and winter as 1/15 per day. However, rainfall data are not given in the paper. Levine and Anderson (1973) showed the importance of rain for the process of faecal decay but noted that even 99mm of rain was not enough to make

sheep pellets fall apart completely. Apart from the potential mechanical 'wash out' effect of rainfall it appears that moisture is a pre-requisite for other processes to take place. From the data presented by Niezen *et al.* (1998) it emerges that, on average, faecal matter disappeared in approximately one week if in the week before 10-15mm of rain had fallen. From Niven *et al.* (2002) it appears that 14 mm of rain was enough for the decay process to take place. Therefore, faecal decay was modelled stochastically, as follows:

If $N \geq 13$ then $d_d = 1/7$ per day (with $N = \Sigma$ mm rain from day of deposition of pellet). This assumes that it does not matter whether the 13 mm of rain falls in one shower or in several smaller spells of rain. It is acknowledged that evaporation rates will vary and thus that in summer more rain may be needed than in winter. However, it seems unlikely that such levels of complexity are needed for a simple comparison of three regions in the UK.

- *Development of eggs in soil (d_s)*

Once incorporated in soil on D_0, the contribution of one day to the total of development, at mean daily temperature T, was modelled as a function of temperature by dividing 1 by the number of days taken to development of 50% of the eggs at temperature T. The relation of $d_{s(T)}$ and T is given by

$d_{s(T)} = 0.00389 + 0.00126 * T$ (with $11.5 \leq T \leq 28$; see chapter 4) Equation 7.1

Development was assumed to be completed if $\Sigma\, d_{s(T)} \geq 1$ and the first day on which this occurred was recorded.

(ii) To determine the cohort cut-off point the following were determined:

- *Start of the autumn hatch*

For each year, after the summer, the 8th day at which the temperatures remained within the hatching threshold was recorded. The 8th day was chosen, as opposed to the 1st day,

to mark the real start of the hatch and to avoid falsely marking a single day with temperatures within the hatching threshold as the start of the hatching peak.

• *Development of eggs prior to the autumn peak*

The development time of eggs before the autumn peak was determined backwards from the start of the autumn peak, as described under 'development of eggs in soil', and the day development would have had to start in order to be ready for the start of the autumn peak (D_p)determined.

• *Incorporation of eggs in soil during summer*

From D_p seven days were deducted for incorporation into the soil and from there, backwards, the date determined at which 13 mm of rainfall had fallen. The day at which this occurred was marked as the cut-off point for eggs to be able to complete development in time for the autumn peak.

(iii) The developmental phase of eggs produced during the autumn peak.

The peak day of the autumn hatch was determined and the process described for the spring-produced eggs repeated for eggs shed at pasture during the autumn peak. The day at which development was completed was recorded.

The soil incorporation and development model were validated against the data presented by Gibson and Everett (1981). In their study monthly series of eggs were put out at pasture and their development followed fortnightly. Weekly temperature and rainfall data were published and therefore, for each week, the mean weekly temperature was modelled

for 7 days while it was assumed that all dung would be incorporated in soil in one week if, in the previous week, ≥ 13mm of rain had fallen.

For model runs on the 30-year weather station data, recorded days of the year were given a number (1 for January 1st, etc.). The predicted days of peak egg output for each year, the cut-off date, the length of the development season, the timing of the autumn peak and the timing of the completion of egg development of eggs deposited during an autumn peak were all compared in a one-way ANOVAs with factor weather station, and Tukey's pairwise comparison. Time taken for egg incorporation in the soil and the time taken for development of eggs after spring peak and just before the cut-off point (during the summer) were compared in two-way ANOVAs with factors weather station and season (spring and summer). The Bonferroni-adjusted significance level was set at 0.007. In order to determine whether number of spring/summer-deposited eggs being able to contribute to an autumn hatch has increased in recent years, for the 30 years under study, the day of the peak spring egg production was deducted from the cut-off point, and the results tested for correlation with time using Spearman's correlation coefficient. As three tests were conducted the significance level was set at 0.017. To find the year a correlation became significant, when a significant correlation was found, the last year was omitted from the analysis, which was then repeated (as described in chapter 2).

Hatching

Regional differences in temperature ranges

Transformed (see Appendix) 30-year mean maximum and minimum daily temperatures were computed from data derived from the three weather stations described in chapter 3.

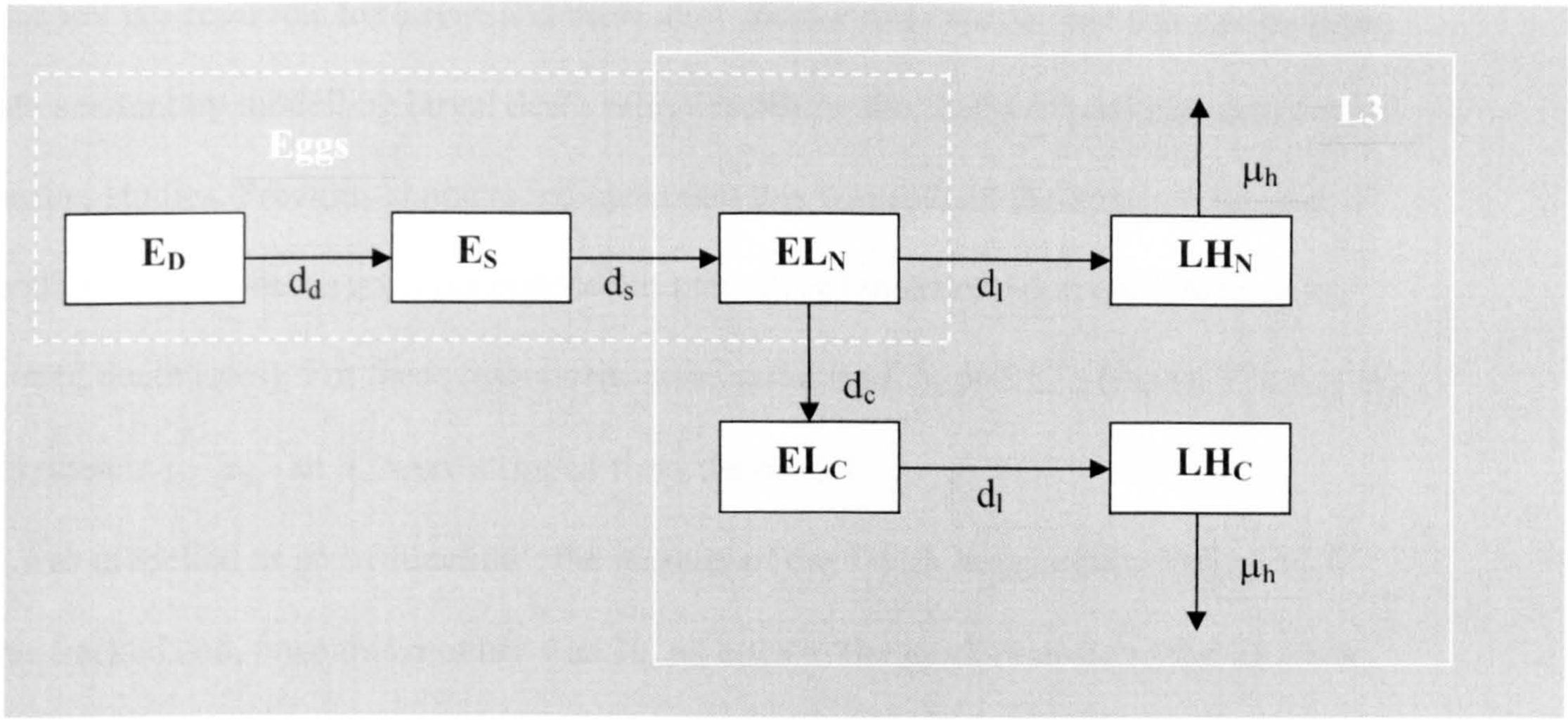


Figure 7.2 Abbreviation of the model of the free-living stages of *N.battus*

Daily minimum values were subtracted from the corresponding maximum values. These temperature ranges were tested for differences between regions in a Kruskal-Wallis test with Mann-Whitney post-hoc tests. As all spring hatching in the three regions took place in this time frame, to test for differences in the spring, April 1st to June 30th were analysed. For the same reason, for Autumn differences, September 1st to November 30th were analysed. A Bonferroni correction was applied and therefore all results are reported at the 0.008 level of significance.

Hatching patterns and disease risk

Parameters

From chapter 6 it emerged that the proportion of larvae migrating onto (and off) herbage, at more or less constant temperatures, is constant within 24 hours of a hatch. It would be important to model this proportion in a full-cycle model but, as hatching is a highly synchronised process, invariably taking place around the same temperature ranges, the migration process is not expected to influence the shape of a peak of larval emergence.

The soil is a reservoir for larvae and these may emerge over weeks, but this can be taken into account by modelling larval death rates describing the shape of peaks determined in pasture studies. Previous chapters indicated that this was indeed the appropriate way of modelling larval death rates (as opposed to modelling laboratory-derived temperature related death rates). For these reasons the state variables $L3_N$ and $L3_C$ (figure 7.1) and the parameters $\mu_{L'}$, $\mu_{L''}$ an d_m were stripped from the model.

d_c was modelled as an 'if function': the number of days with temperatures below 11°C was tracked and, once this number was 30, all eggs in the model moved to the EL_C box. Initial lower and upper temperature thresholds for hatching stimulus were set at 11°C and 17°C. With regards to the parameter d_l, it was assumed that all eggs hatched after 16 days of within-threshold temperatures, no eggs hatching in the first 8 days and all eggs hatching, in a linear fashion, over the following 8 days. As eggs had been shown to halt their hatch when the temperature moved out of the hatching range but also to resume hatching when placed back at within-threshold temperatures it was assumed that eggs had a 'memory' of within-threshold temperature experience. This memory was set at three weeks. The larval death rate on herbage (μ_h) was extracted from the pasture studies of larval peaks presented by Gibson (1959b) Thomas (1959b) Thomas and Stevens (1960), Boag and Thomas (1975) and Graham, Harris and Ollerenshaw (1984). The maximum peak herbage count of all studies was calibrated at 1000 L3/kg DM and the values after the peak recalculated from this point. Values of larvae/kg DM and corresponding time were fitted to the model $Larvae_{(t)} = Larvae_{(0)} * e^{-\mu t}$ using maximum likelihood estimation (MLE).

The hatching model was run stochastically, with time steps of one day, for the Southwest and for Scotland. Every simulation used 10,000 repetitions. Minimum temperatures were

modelled as the normal distribution of 30-year daily minimum temperatures derived from weather stations. Minimum and maximum daily temperatures are likely to be correlated and therefore stochastic modelling of maximum temperatures in the same fashion may lead to unrealistic temperature ranges. Therefore, for each year, the minimum temperature was deducted from the maximum temperature and the normal distribution of the range determined. Maximum daily temperature was now modelled as the simulated minimum temperature plus the simulated range. In modelling exercises, where needed, simulated maximum temperatures were increased by increasing the mean range by 1 or 2°C. The model was run for the cohort of eggs ready in August. If eggs did not manage to hatch in autumn they were carried over to the next year. The proportion of eggs hatching without chilling was simulated as the proportion of total eggs available in the depot in august allowed to hatch in the first hatching opportunity.

The timing of the maximum magnitude of each larval peak was recorded, as well as the proportions of available eggs hatching in each autumn or spring. A risk parameter (D_r) was devised and recorded. It was assumed that the disease risk presented by larval peaks was a function of the magnitude of the peak and the probability of lambs encountering the peak at pasture. However, lambs would only get sick if the level of larval presence was over a certain threshold. Therefore, $D_r = M * d_{40}$, with M = the magnitude of the peak at the highest point and d_{40} the number of days during which a proportion of 0.40 of the number of larvae hatching during that peak was present at pasture (i.e. if eggs hatch gradually and at no point more than 40% of the depot hatching is present at pasture D_r =0). The proportional cut-off point of 0.40 was chosen by running the model 200 times and observing the shapes of peaks. D_r was determined by modelling the hatching of one egg. Variables, equations and parameters used in the models are summarised in tables 7.3 and 7.4.

The hatching model was validated in spring 2006 and 2007, on pasture where, in the year before, peaks of *N. battus* egg output had been registered (see section 7.2). Weekly, these pasture plots were sampled walking over the plots in a large W-shape. At each tenth step a herbage sample was taken to the front, to the left and to the right. The herbage was plucked at approximately 2cm above soil level and care was taken not to include any soil in the sample. After the W-shape had been completed the process was repeated while walking back to the starting point. Thus, 2 samples of approximately 500 grams of herbage were plucked. The samples were processed as described in chapter 6 but 40 litres of water was added to the tubs.

Abbreviation	Variable	Equation
E_D	Eggs in dung	$dE_D/dt = -E_D\, d_d + E_D$
E_S	Eggs in soil	$dEs/dt = -E_S\, d_s + E_D\, d_d$
EL_N	Embryonated eggs (non-chill)	$dEL_N/dt = -EL_N\,(d_c + d_l) + E_S\, d_s$
EL_C	Embryonated eggs (chilled)	$dEL_C/dt = -ELc\, d_l + EL_N\, d_c$
LH_N / LH_C	Hatched larvae (non-chill and chill) on herbage	$dLH_{(N+C)}/dt = -(LH_N + LH_C)\, e^{-\mu h} + (EL_N + EL_C)\, d_l$

Table 7.3 State variables used in the models of *N. battus* ecology. d_c and d_l never have a value (table 7.4) at the same moment in time

Abbreviation	Parameter	Value	Dependent on
d_d	Decay rate of dung	1	Σ_{rainfall} ($t_{(\text{egg deposited})}$-$t_{-8}$) ≥ 13 mm
d_s	Development rate in soil	1	$\Sigma\, d_{s(T)} \geq 1$, with $d_{s(T)} = 0.00389 + 0.00126 * T_{(t)}$ *AND* $11.5 \leq T_{(t)} \leq 28$°C
d_c	Chilling rate	1	$\Sigma_{\text{chill days}} \geq 30$; Chill day = T < 11°C
d_l	Hatching rate	0.125	$\Sigma_{\text{hatch days}}(t_{-22}$-$t_{-1}) = 8$ *AND* t = hatch day; Hatch day = $11 \leq T \leq 17$°C
μ_h	Larval death rate (herbage)	0.055 (0.045-0.076)	-

Table 7.4 Parameters used in the models of *N. battus* ecology. 'Dependent on' means that the parameters only have the value if the condition holds true. For μ_h the 95% confidence intervals are given.

7.1.3 Model output

Development

Validation

The results of the validation of the development model are given in table 7.5. Very good agreement was found between 'detected' and 'predicted' dates for all but those eggs put out during the months July and August, overestimating development of the former while somewhat underestimating development of the latter .

Regional differences in egg deposition and egg development

Model output for the three weather stations is summarised in table 7.6, which only gives the results of ANOVAs comparing the three regions. In all years/regions but 1977 in Scotland, eggs produced during the spring peak were predicted to be fully developed

Plot seeded (1973) *	L3 detected *	L3 predicted
04/01	01/07	16/06
01/02	23/07	16/06
01/03	02/07	16/06
24/03	26/06	25/06
20/04	22/06	20/06
22/05	23/07	13/07
19/06	31/07	07/08
18/07	some Nov./Dec. 1973 majority May 1974	19/09
19/08	some Nov./Dec. 1973 majority May 1974	24/05/1974
18/09	May 1974	24/05/1974
13/10	10/06/1974	11/06/1974
20/11	10/06/1974	15/06/1974
15/12	10/06/1974	15/06/1974

Table 7.5 Validation of the development model. Dates are given as day/month of the year; all dates in 1973 unless stated. * Data extracted from Gibson and Everett (1981). Plots were checked fortnightly and therefore 'L3 detected' days may be up to 13 days late.

before the start of the autumn peak. Eggs produced during an autumn hatch never reached advanced stages of development before a following spring hatch but were predicted to be always fully developed before the next autumn hatch, one year later. Assuming the weather stations are representative of the regions, the spring hatches were significantly later in Scotland than in the two other regions while the autumn hatches started significantly earlier, resulting in a significantly shorter development season. The length of this season increased significantly over the 30 years in Scotland ($r_s = 0.696$, $p < 0.001$; year = 1995) and in the Midlands ($r_s = 0.690$, $p < 0.001$, year = 1997) but not in the Southwest ($r_s = 0.373$, $p = 0.043$). The predicted cut-off dates are significantly earlier in Scotland but, between the regions, are within weeks of each other.

Soil incorporation days and time taken for development of eggs were predicted not to differ between regions (table 7.6). Incorporation of eggs in soil was also not significantly faster in spring than during the late summer (i.e. between the cut-off point and the autumn hatch; $F_{(1,179)}$ =3.9, p = 0.050). However, time taken for development of eggs was predicted to be significantly faster in spring than in late summer ($F_{(1,179)}$ = 104.6, p < 0.001).

	peak egg output (spring)	soil incorp. (spring)	dev. time 1 (spring)	dev. completed	cut-off date	dev. season (peak to cut-off)	soil incorp. (cut-off)	dev. time 2 (cut-off)	start autumn peak	autumn eggs ready (year 2)
Yeovilton										
mean	30May	13	40	21 July	19 Aug.	81	14	49	21 Oct.	25 June
sd	11.5	5.2	5.9	11.4	10.2	15.2	6.0	7.2	13.8	8.1
min	23 April	8	31	3 July	2 Aug.	48	8	42	25 Sept.	12 June
max	17 June	32	67	23 Aug	8 Sept.	117	31	73	18 Nov.	9 July
Waddington										
mean	6 June	15	39	30 July	16 Aug.	72	12	48	14 Oct.	29 June
sd	12.1	7.7	3.0	13.5	5.7	13.5	5.7	7.2	10.1	14.9
min	12 May	8	32	7 July	28 July	54	8	40	21 Sept.	7 June
max	27 June	35	44	29 July	4 Sept.	68	29	45	1 Nov.	17 July
Paisley										
mean	16 June	13	40	8 Aug.	5 Aug.	49	9	44	27 Sept.	30 June
sd	9.9	6.1	1.9	13.3	7.9	13.2	4.5	3.5	8.5	7.7
min	29 May	8	36	18 July	23 July	19	8	38	12 Sept.	15 June
max	11 July	32	42	18 Sept.	20 Aug.	70	27	55	13 Oct.	18 July
ANOVA	$F_{(2,89)} =$ 18.5, $p <$ 0.001	$F_{(2,179)} =$ 2.9, $p =$ 0.074	$F_{(2,179)} =$ 2.9, $p =$ 0.060	-	$F_{(2,89)} =$ 19.1, $p <$ 0.001	$F_{(2,89)} =$ 39.3, $p <$ 0.001	see soil incorp. (spring)	see dev. time 1	$F_{(2,89)} =$ 18.5, $p <$ 0.001	$F_{(2,89)} =$ 2.9, $p =$ 0.060
Tukey	Sc-Sw & Sc-M: $p < 0.001$	-	-	-	Sc-Sw & Sc-M: $p < 0.001$	Sc-Sw & Sc-M: $p < 0.001$	-	-	Sc-Sw & Sc-M: $p < 0.001$	-

Table 7.6 Regional differences in egg development characteristics, 1977-2006. Soil incorporation = days taken for eggs to be incorporated in soil; Development time = days taken to complete egg development in soil; Cut-off date = day after which eggs deposited at pasture are predicted not to complete development before the autumn hatching window; Development season = days between the peak output of eggs in spring and the cut-off date. Only significant Tukey comparisons are given; Sc = Scotland, Sw = Southwest, M = Midlands.

Hatching

Regional differences in temperature ranges

Regional 30-year daily mean maximum and minimum temperatures are illustrated in figure 7.3. Minimum temperatures are virtually identical for the three regions whereas there are differences in maximum temperatures. This results in wider ranges between minimum and maximum daily temperatures in the warmer Southwest than in the other two regions (as is illustrated for the Southwest and Scotland in figure 7.4). For all three regions, ranges appear smaller in the autumn than in spring. Temperature ranges were, during both spring and autumn hatching months, shown to differ significantly between the three regions (table 7.7). In spring, ranges were significantly greater in the Southwest

	Kruskal-Wallis test	Mann-Whitney post-hoc tests
Spring	$H_{(2)} = 40.5$, $p < 0.001$	**Sw- M:** $U = 57221$, $r = -0.35$, $p = 0.001$ **Sw- Sc:** $U = 51992$, $r = -0.54$, $p < 0.001$ **M - Sc:** $U = 62250$, $r = -0.16$, $p = 0.127$
Autumn	$H_{(2)} = 67.1$, $p < 0.001$	**Sw- M:** $U = 2961$, $r = -0.35$, $p = 0.001$ **Sw- Sc:** $U = 1222$, $r = -0.86$, $p < 0.001$ **M - Sc:** $U = 2475$, $r = -0.49$, $p < 0.001$

Table 7.7 Differences in spring and autumn temperature ranges between the Southwest (Sw), the Midlands (M), and Scotland (Sc). N = 91 days.

than in both other regions, while no differences were detected between the North Midlands and Scotland. In autumn ranges were significantly greater in the Southwest than in the North Midlands and, significantly greater in the North Midlands than in Scotland.

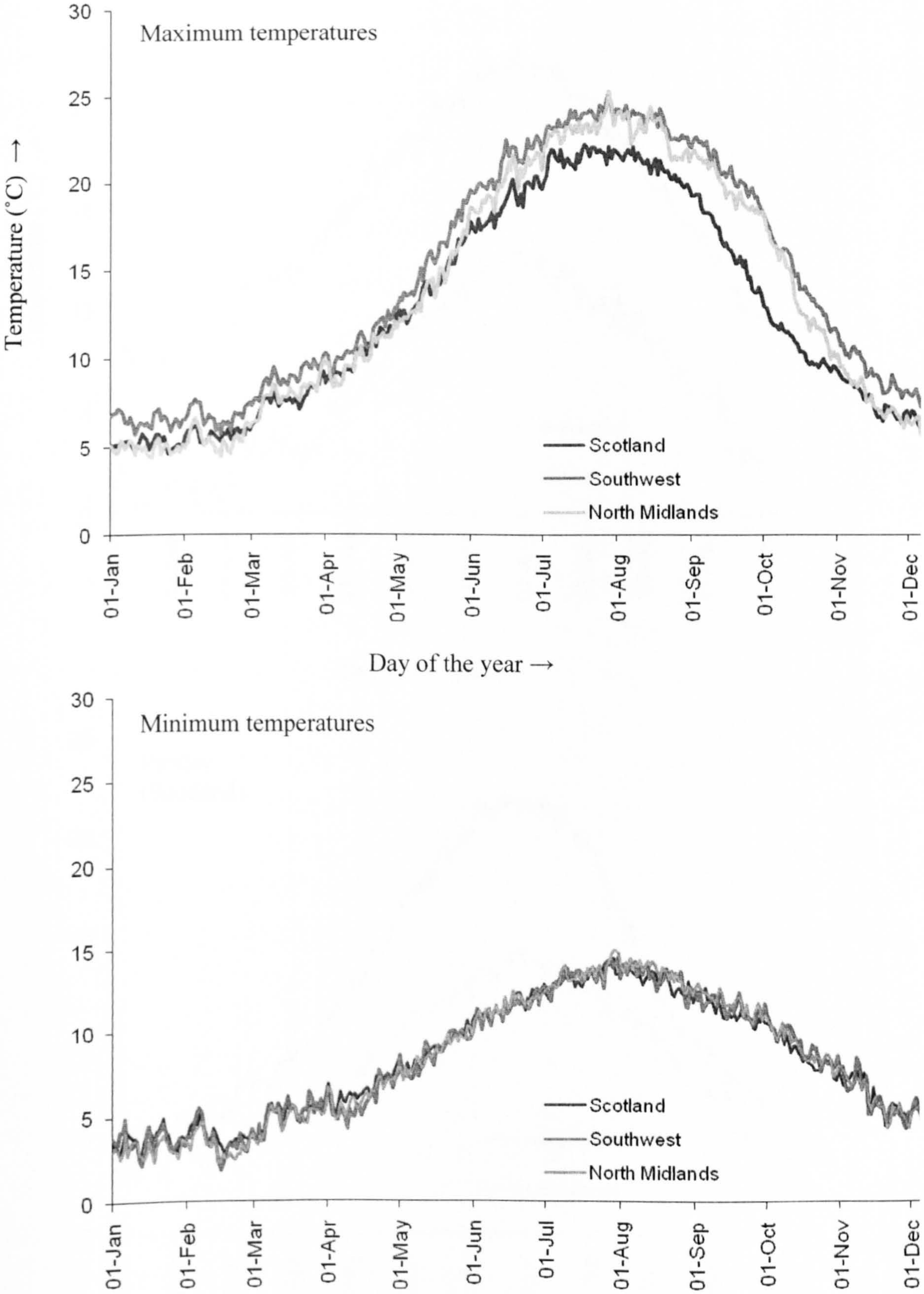


Figure 7.3 30-year mean daily maximum and minimum temperatures in Scotland, the Southwest and the North Midlands.

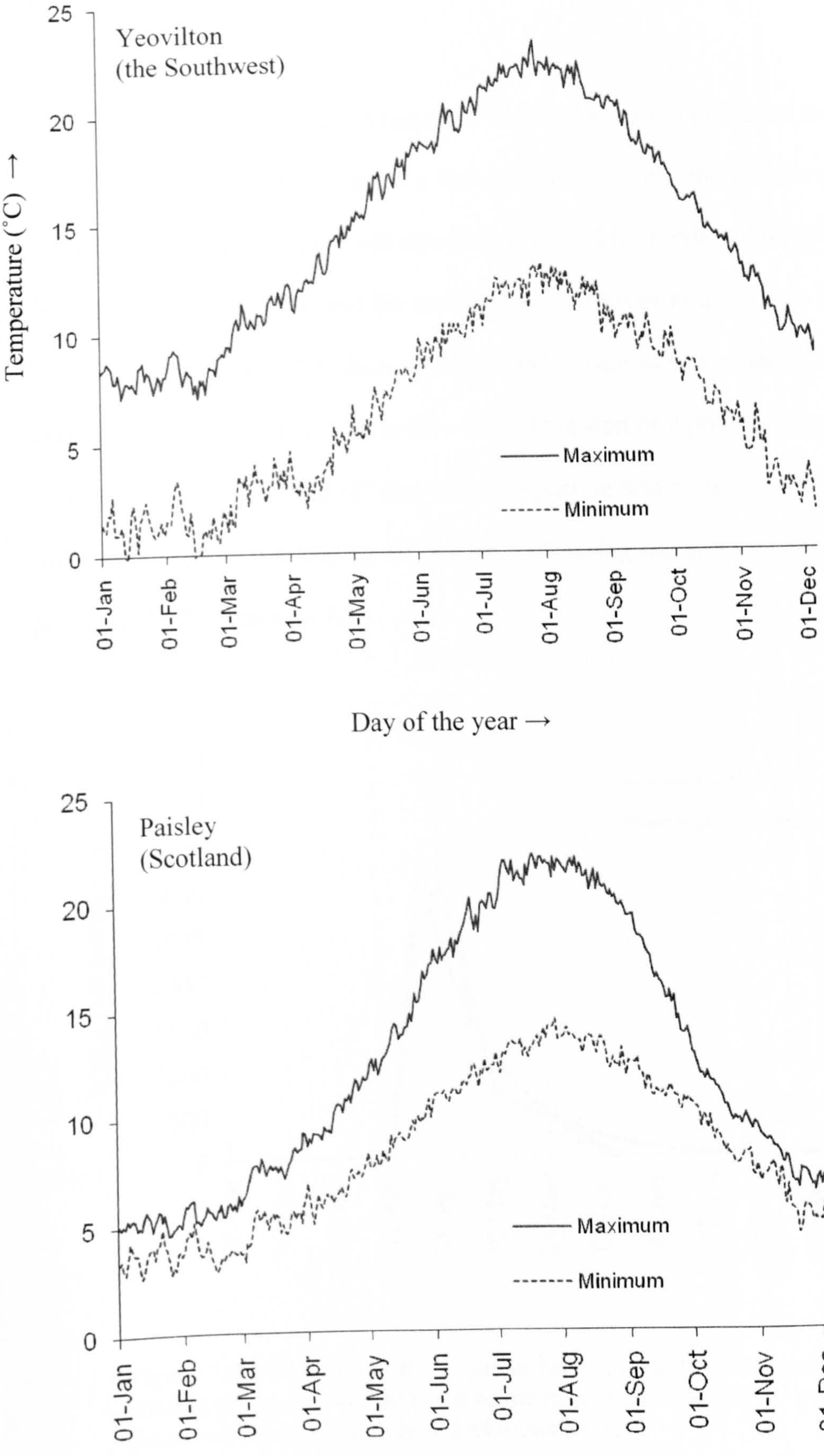


Figure 7.4 30-year mean daily temperature ranges in the Southwest and Scotland.

Validation

At a lower hatching temperature threshold of 11°C the model predicted the peak to occur approximately one week late, but at a lower threshold of 9°C there was a very close agreement between measured and modelled values. The overlay of the peak constructed from the grass plucks 2006 and the modelled peak is given in figure 7.5. In 2007, mean larval counts of up to 534 *N. battus* L3/ kg DM herbage were encountered at the start of March and counts gradually fell to zero around the start of April. Herbage counts remained zero up to the end of June, when the pasture was mown, and fertilised, and the study had to be aborted. The model, run on 2007 spring soil surface temperatures, predicted the first hatch in July.

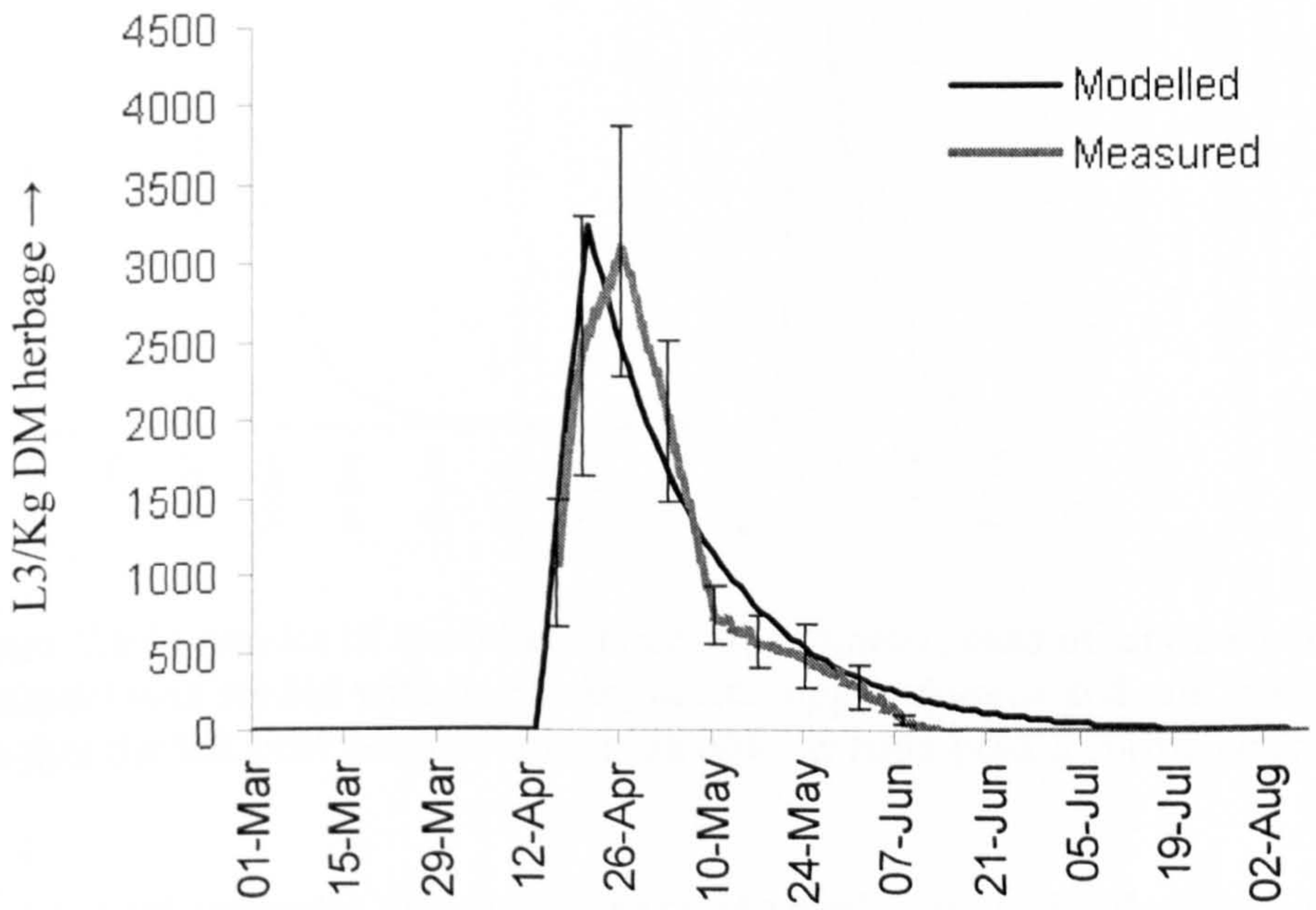
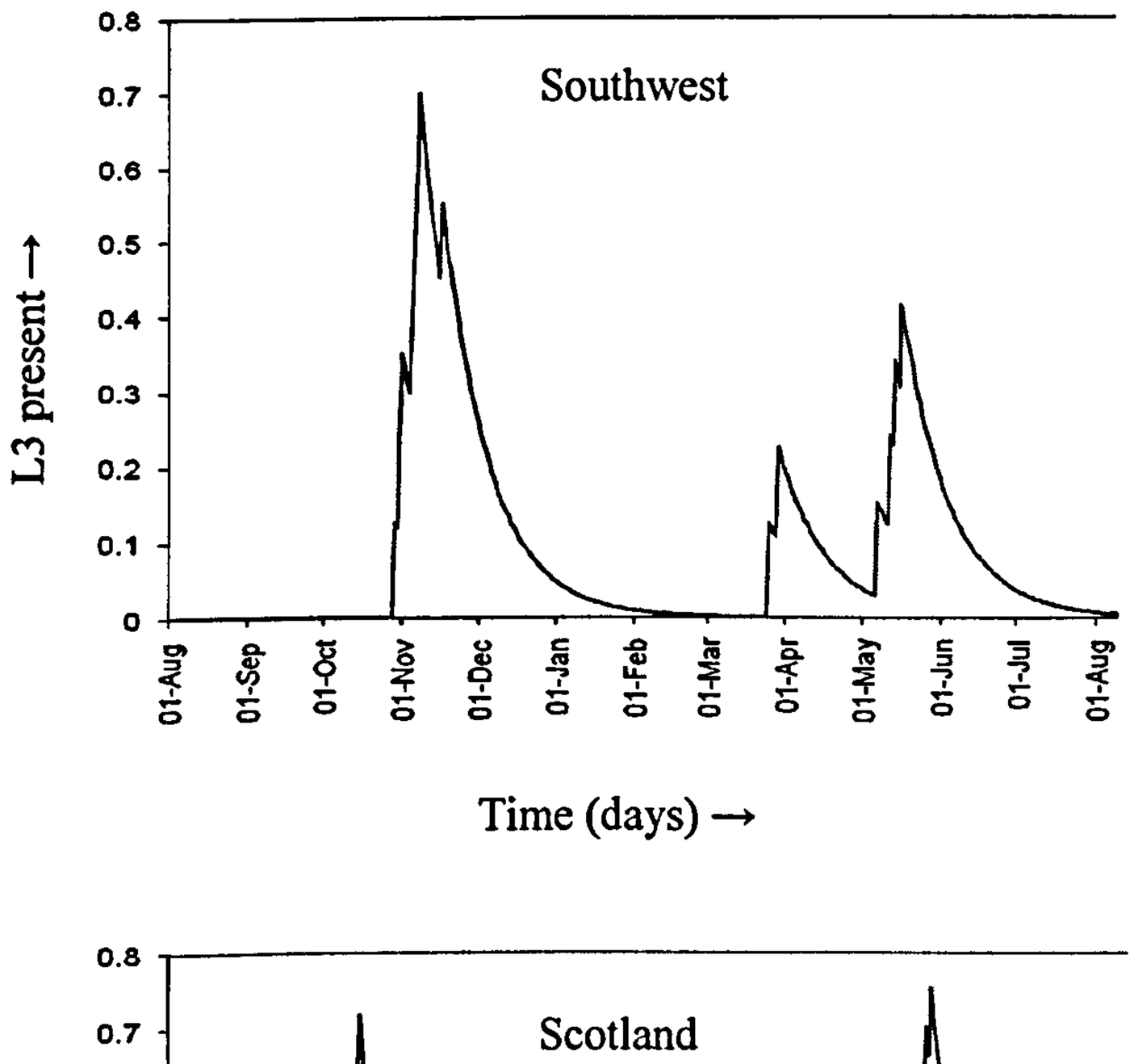


Figure 7.5 Validation of the *N.battus* hatching model. The number of eggs seeding the model was adjusted to the maximum value of the grass pluck peak. Error bars represent standard deviations (n =2).



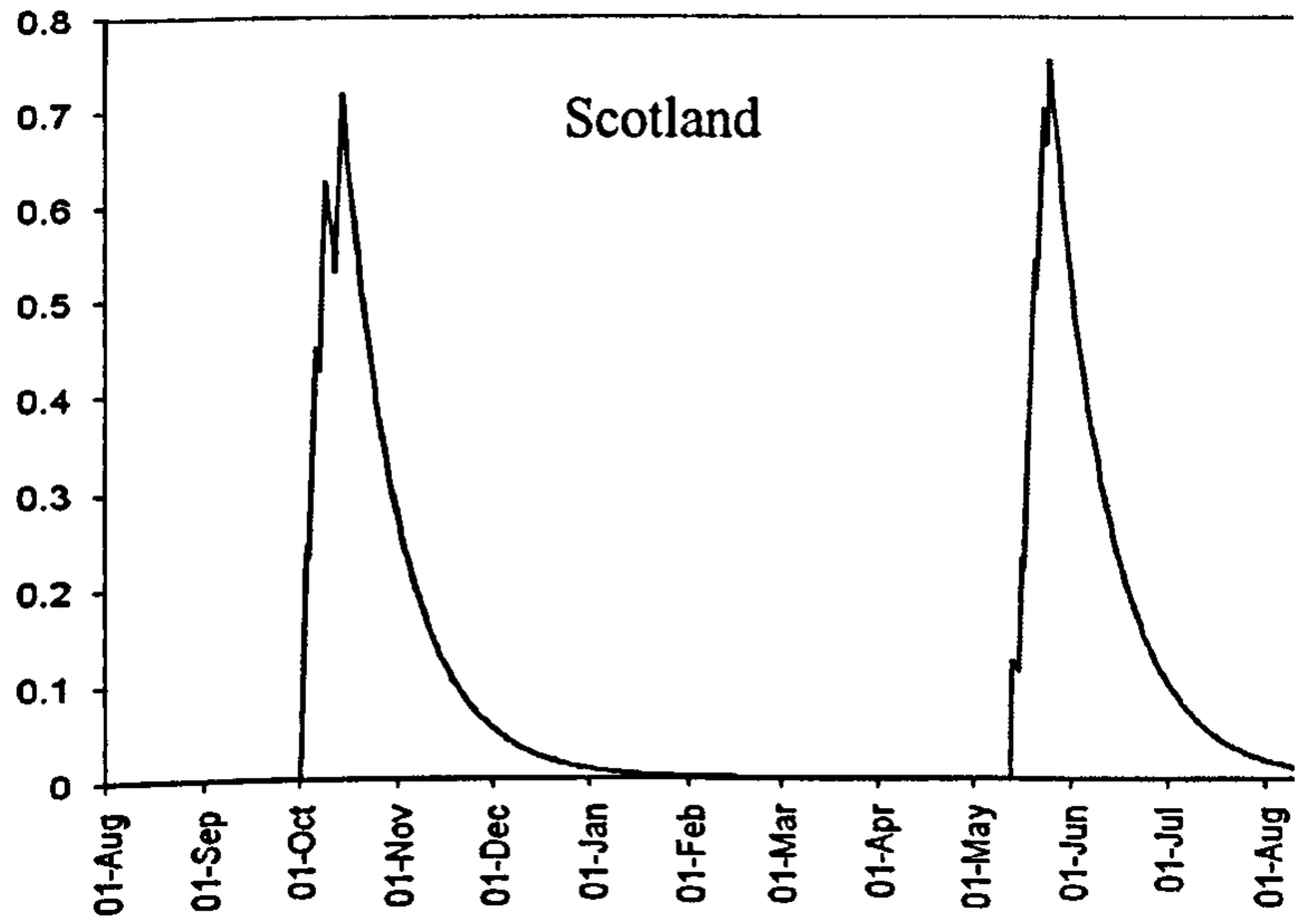


Figure 7.6 Examples of modelled shapes of *N.battus* peaks of larval emergence. Here, the model was seeded with one embryonated egg in August and one in February. Please note that the left peak is an autumn peak and the right peak a spring peak.

Some typical examples of autumn and spring peaks, in the Southwest and in Scotland, are given in figure 7.6. In the Southwest, especially in spring, 'split peaks' were predicted to occur regularly (the effect of which is quantified below). The hatch would be initiated, but not completed, while one or more peaks would occur later in the year, at which time the level of L3 from the first peak had fallen to low levels. The magnitude of

levels of L3 at pasture during the peaks was normally higher in autumn hatches, which took place over a shorter period of time. In Scotland split peaks did occur but the rapid emergence of the total pool of larvae over a short period of time was observed to be the norm, both in spring and in autumn.

Risk (D_r)

Yeovilton	Present	+1°C	+2 °C	**Paisley**	Present	+1°C	+2 °C
Spring	5.3 (0-13.4)	3.2 (0-12.6)	1.5 (0-11.1)		10.8 (1.8-14.3)	9.2 (0-14.3)	7.7 (0-14.1)
Autumn	10.3 (0-14.3)	8.8 (0-14.1)	7.2 (0-14.0)		13.4 (10.6-15.0)	13.0 (9.2-14.9)	12.9 (8.1-14.9)

Timing

Yeovilton	Present	+1°C	+2 °C	**Paisley**	Present	+1°C	+2 °C
Spring	May 15th (689)	May 3rd (1129)	April 15th (1453)		May 30th (337)	June 1st (1091)	June 15th (4001)
Autumn	Nov. 3rd (113)	Nov. 9th (195)	Nov. 12th (370)		Sept. 30th (25)	Oct. 9th (36)	Oct. 12th (41)

Table 7.8 Mean predicted risk and timing of peaks of larval emergence in the Southwest (Yeovilton) and in Scotland (Paisley). For the risk parameter D_r the 95% confidence intervals are given between brackets, for 'timing' the variance.

The model predicts, for the current situation, the magnitude of the mean risk parameter in the Southwest to be approximately half that of Scotland (table 7.8). However, the upper confidence limits are similar. For both regions mean risk potential is higher in autumn than in spring. If temperature ranges are extended by 1 or 2°C the risk parameter falls in both regions, taking predicted relative disease risk in the Southwest to very low levels. However, for both regions, the upper confidence limits remain at high levels. Also, in

both regions, the risk potential from autumn peaks remains higher than that of spring peaks.

The variance around the mean predicted peak hatching date is twice as high in the Southwest as in Scotland. If temperature ranges are increased in 1°C steps the variance less than doubles in the Southwest but quadruples in Scotland. The variance around autumn peaks is much lower than that around spring peaks for both regions and remains at low levels when temperature ranges are increased.

The hatching of non-chilled eggs

In the Southwest, the lower 95% confidence limits of eggs hatch only after chilling touch the zero line (figure 7.7), indicating that in some years no hatch may occur. Mean probabilities of hatching steadily increase with each increment of proportion of non-chill hatch. Lower 95% confidence limits, decrease in a similar fashion, but only up to a level of 0.6-0.7, when the lower confidence limit does not decrease any further. In Scotland, the mean probability runs at very high levels regardless of the proportion of eggs hatching as non-chilled eggs. Increasing this proportion shortens the lower 95% confidence limits somewhat but only marginally and not consistently between increments.

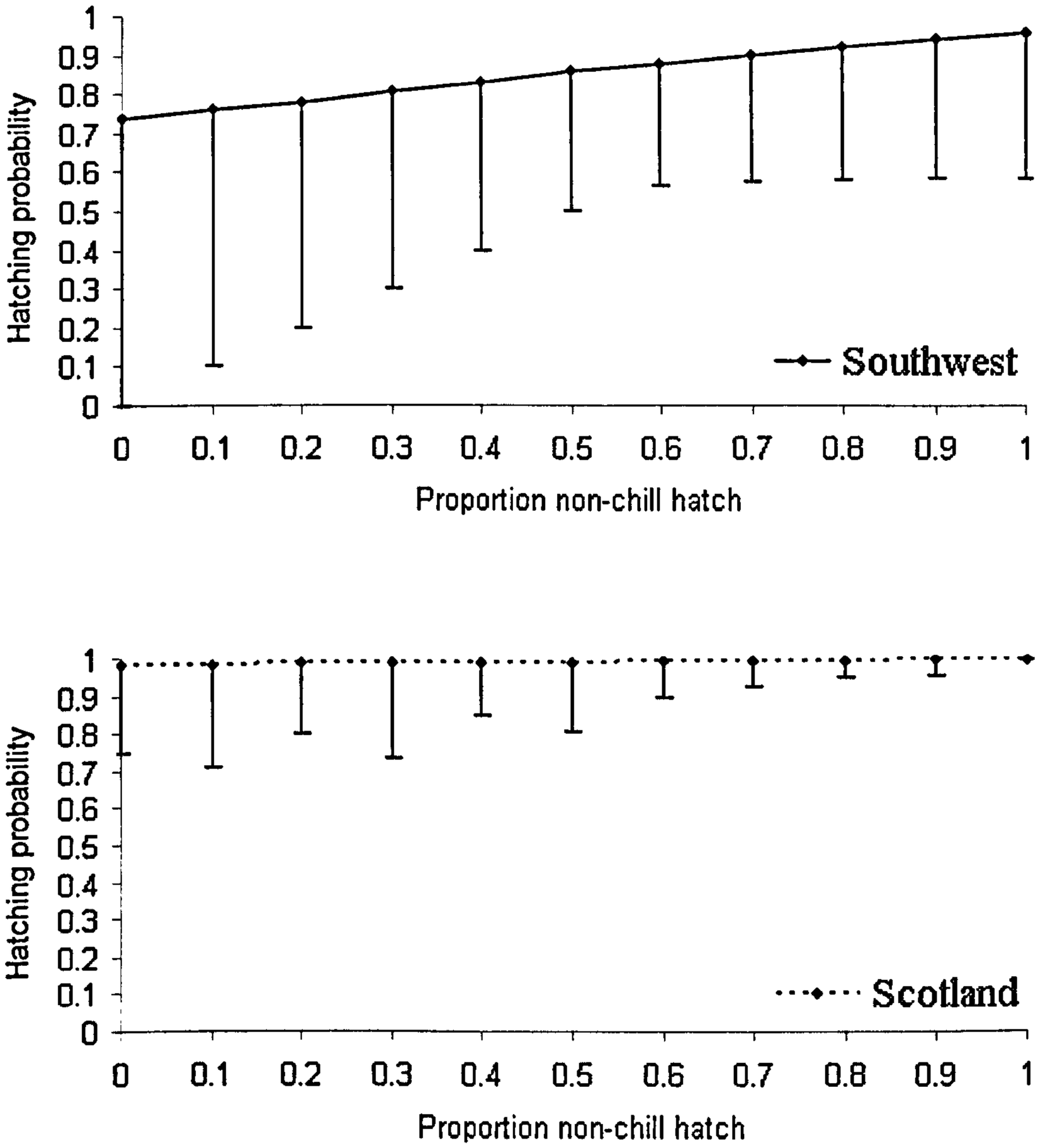


Figure 7.7 The influence of the proportion of eggs hatching without chilling on the probability of hatching within one year (one spring and one autumn peak) in the Southwest and in Scotland. Error bars represent 95% confidence intervals. In the Southwest, the bar of the lower 95% confidence limit of the zero proportion non-chill hatch scenario overlaps the x-axis.

In the Southwest, at lower proportions of non-chill hatch, the probability of hatching is considerably increased by 1°C step increases in upper hatching threshold (figure 7.8). The benefit of increasing the upper limit for hatching to the probability of hatching is always predicted to be greater than increasing the proportion of non-chilled hatch. However, the lines representing different thresholds are converging and at higher proportions of non-chilled hatch the benefits of a higher threshold are much smaller than at lower proportions of non-chill hatch. Already at an upper threshold of 18°C, it appears

that there are virtually no benefits of increasing the proportion of hatching without chilling to hatchability.

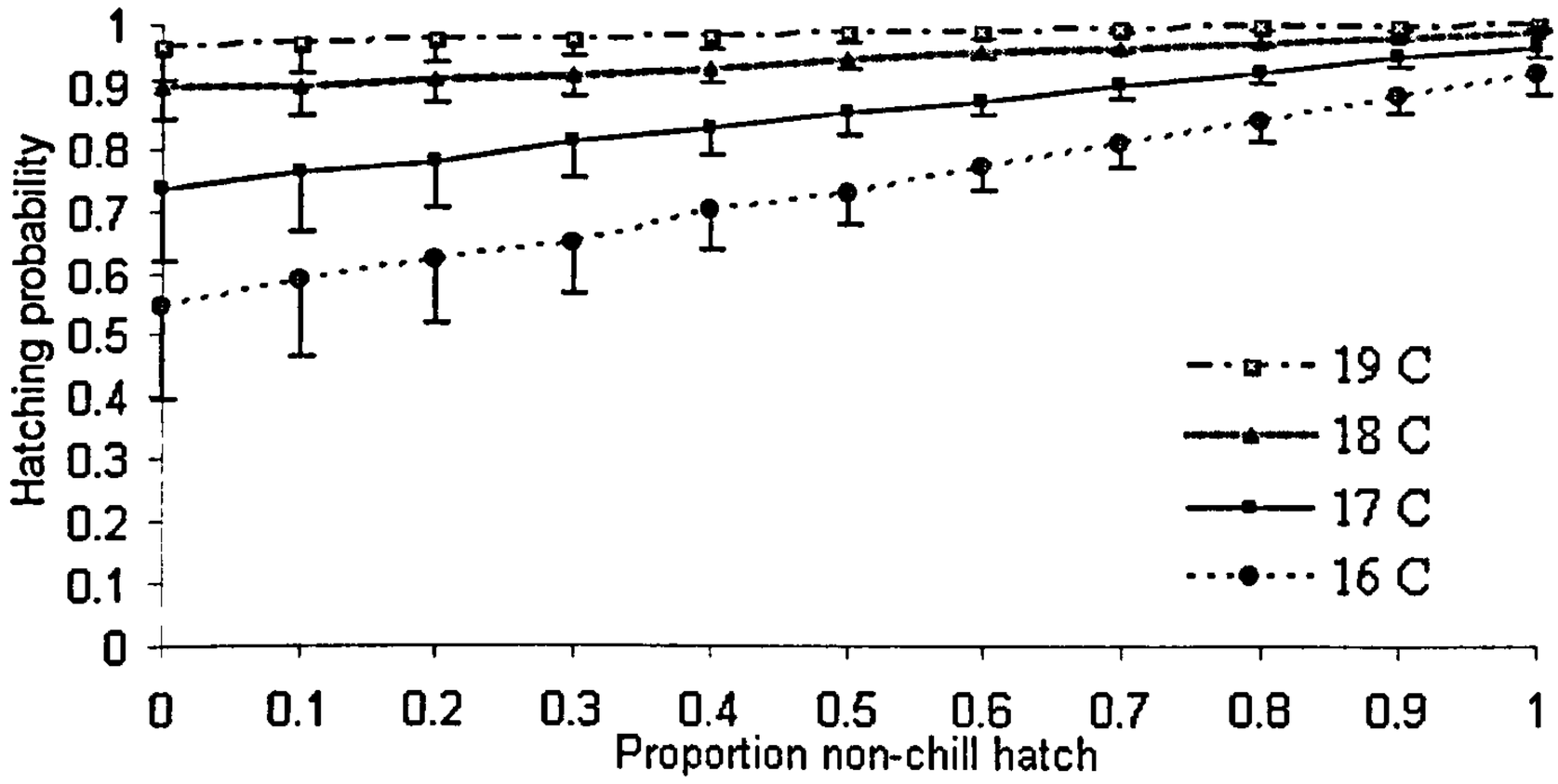


Figure 7.8 The influence of changes to the upper hatching threshold on the probability of hatching within one year (one spring and one autumn peak) in the Southwest. Error bars represent standard deviations.

7.1.4 Discussion

Egg development

When validated against field data the model appears to predict the completion of egg development very well for all but the summer months. In the study by Gibson and Everett (1981), egg deposition in July was followed by a fortnight with rainfall below the 13mm threshold and this would suggest that the model under-estimates the effects of prolonged desiccation. However, egg deposition in August was followed by 5 weeks of drought and some of these eggs had developed to the L3 stage earlier than predicted. The latter may be related to mean temperatures rising from 15 to 20°C over the course of three weeks following egg deposition and sharply falling temperatures after these three

weeks (chapter 4). As, for the model, the completion of development of 50% of the eggs was used, it may also just reflect variability in egg populations. If egg development during the hottest months of the year is somewhat unpredictable this suggests that the model may not be able to predict the egg cohort cut-off point with great accuracy. At pasture it was shown (see section 7.2) that the egg output of lambs during these months is at their lowest point. Therefore, even if the real cut-off point is a few weeks earlier or later this is unlikely to have serious implications for the prediction of the risk to animals from subsequent peaks of larval emergence.

Eggs deposited during spring were predicted to always be fully developed by the end of summer while autumn-deposited eggs were never predicted to contribute to the following spring peak. In a cohort-based model, the total egg output of one year, i.e. the time between two cut-off dates, can be modelled as one cohort ready to hatch in autumn and/or the following spring. None of the eggs within this cohort will have chilling experience. A stochastic, individual-based, model can simply model completion of UK egg development by the normal distributions of spring hatch, soil incorporation, development time in spring and summer presented here. Eggs completing development before the autumn hatch would then be included in the autumn hatch depot while eggs not doing so would be included in the depot of eggs ready for hatching the following autumn. Such a model would make false predictions only for eggs that complete their development during the autumn hatching window. These will invariably be eggs that have been deposited during the summer months and their (relative) number will be low.

Although the within-year egg development at pasture may follow predictable patterns, the origin of individual eggs within a pool, contributing to any given peak of larval

emergence at pasture, is not easily predicted. Against a background of proportions of eggs hatching without, or only after, chilling the above means that, during each spring hatching window, the following eggs may hatch: 1) a proportion of eggs deposited in the previous spring, 2) a proportion of eggs deposited in the autumn the year before last and 3) all eggs that were not able to hatch in the most recent autumn hatching window of opportunity. An autumn hatching opportunity may see the following eggs hatch: 1) a proportion of eggs deposited in the spring of the same year, 2) a proportion of eggs deposited in the previous autumn and 3) all eggs that were ready, but not able to hatch, in the most recent spring window of opportunity. The complexity of the history of larvae contributing to any peak of larval emergence may be an important reason why the strategy of avoidance of grazing of pasture grazed by lambs in the previous spring (Black, 1959) has far from rapidly eradicated the parasite.

The time frame during which eggs deposited at pasture in spring or summer would be able to contribute to the next autumn or spring hatch was significantly shorter in Scotland. Eggs hatching only after chilling will, if not fully developed in the autumn of the year of deposition, incur a full year delay in emergence from the egg. If the climatic data from the three weather stations is representative for the regions then it can be concluded that temperature influences on egg development cannot account for the relative success of the species in Scotland (chapter 2). However, in recent years, the 'development season' has lengthened very significantly in Scotland. Therefore, extra eggs contributing to spring peaks may be partially responsible for the recorded rise in disease incidence. As increases in mean temperature are not likely to increase egg death significantly and are not beneficial to egg development rates (chapter 4; present data) this increase in development opportunities of eggs deposited later in the year may be the main

effect of climate change on the developmental stages of *N. battus*. The very long development season, in combination with the lowest incidence rate, in the Southwest shows that this does not have to translate into disease.

Hatching

Surprisingly, mean 30-year minimum temperatures were very similar across the regions. However, maximum temperatures differed considerably. As a result, the daily temperature range was found to be significantly greater in the Southwest, both in spring and in autumn. From this it would be expected that, on average, when soil temperatures rise in spring and the minimum temperature enters the hatching range, the probability of the maximum temperature already exceeding the upper hatching threshold is greater in the Southwest than in Scotland. If the upper hatching threshold were exceeded hatching would be unlikely to occur. For all three regions the probability of this happening would seem greater in spring than in autumn.

The model predicted patterns measured at pasture very well, both in 2006, where a strong spring hatch was recorded and in 2007, which was characterised by an absence of hatching in spring. The plot of pasture sampled in 2007 was the plot where a sharp peak in worm egg counts had been recorded in 2006 (see section 7.2). The presence of *N. battus* on this plot was confirmed at the start of the year. In 2006, the model, when run on 11 and 17°C hatching thresholds, underestimated the hatch but at a 9-17°C range it proved an almost perfect fit of the peak registered at pasture. Although at 9°C no hatching was observed in the laboratory this may be explained by a time lag between the temperature moving out of the hatching range and the halting of the hatching process. In

chapter 4 it was observed that hatching abruptly came to a halt when temperatures rose out of the top end of the range whereas hatching continued for two days when temperatures dropped below the lower threshold. Thus, after a series of days with within-threshold temperatures, a few days where minimum temperature drops somewhat below the threshold may not have an effect on the hatching process.

The validated model subsequently confirmed that the differences in maximum temperature between regions are likely to lead to differences in epidemiology. In spring, in the Southwest, peaks of low magnitude, often in combination with a dispersed hatch of the depot of eggs over several months, were commonly predicted. In Scotland hatches of the total depot of eggs over the minimum time required to complete the hatching process were more common. Simulations of the risk potential parameter, and the timing of the peak of the hatch, in the two regions gave good agreement with observed disease patterns in spring (chapter 2): in the Southwest, disease risk was predicted to be lower while the timing of the peak hatch showed a far wider distribution. The model predicted that the potential for disease as the result of a mass hatch was greater in autumn than in spring. This shows the limitation of a model excluding the host. However, in terms of climate change, it is important to note that the autumn was predicted to be a more reliable outlet for the pool of embryonated eggs in both regions, in particular in the Southwest. Overall disease risk was predicted to decrease with increasing minimum-maximum temperature ranges, especially in spring. However, if the temperature range was widened by a year-round 2°C the risk of disease was still predicted to be higher in Scotland than current risk in the Southwest. Moreover, the upper 95% confidence limits changed little, showing that, although more years with lower disease incidence are likely to be witnessed, the threat of severe disease is likely to remain. If several years of 'non-clinical' egg output

are followed by a year with perfect hatching conditions, the latter may give rise to very severe disease indeed. The timing of the peak was predicted to become far less predictable and thus disease may be witnessed when least suspected. Perhaps surprisingly, the variance in the maximum peak was predicted to increase far more dramatically in Scotland than in the Southwest. Perhaps this can be explained by the more diverging character of the minimum and maximum temperatures around the minimum temperature required for hatching (as opposed to the more parallel boundaries of the range in the Southwest; See figure 7.4). If the range is widened to the point where hatching days become more limited, a wider variation in range makes hatching more unpredictable.

The model showed that there is likely to be pressure on the parasite to increase proportions of eggs hatching without chilling from the climatic environment and these pressures are likely to be much stronger in the Southwest than in Scotland. Interestingly, above a non-chill hatch proportion of 0.7 further increases were predicted to have little extra benefit. Adaptation of the upper threshold for hatching to the climatic environment, by increasing it just one degree Celsius, was predicted to be more advantageous to the hatchability of eggs than any scenario of non-chill hatch. This confirms that the upper threshold for hatching is a critical parameter in the epidemiology of the parasite and stimulates study into variation in this threshold between isolates. Given the proportions of non-chill hatch detected in field isolates the finding may be interpreted as further (see chapter 4) evidence that adaptation of temperature thresholds to new environments is either very slow to occur or does not occur. The alternative hypothesis, that host absence and/or host immunity and/or generation time are stronger driver(s) of hatching behaviour than climatic influences needs investigation.

7.2 A simple full-cycle model of *N.battus* epidemiology

Patterns of larval emergence at pasture may take any shape known to man but the outcome of these infection patterns ultimately depends on the host. *Nematodirus battus* epidemiology may be changing and as a result infections of older, partially immune, animals in spring will be more common. Contributions of these type of infections to the population growth of the parasite have as yet to be quantified. No longitudinal studies detailing egg output of older lambs and ewes have as yet been published. Amongst veterinarians, and veterinary parasitologists, the hypothesis that older animals are refractory to infection, protected by 'age resistance', is widely accepted. However, there is no substantial evidence for this phenomenon (see chapter 1). This part of the chapter makes a start on the investigation of contributions of autumn infections to total annual parasite offspring. The results are then, together with the results of section 7.1, modelled in a simple probabilistic model predicting 10-year *N.battus* offspring, with a view to exploring avenues for on-farm parasite control.

7.2.1 Exploring contributions of autumn infections to total annual egg output

Trichostrongyloid egg counts of individual lambs were, in the three lamb crops of the organic sheep farm described in previous chapters, determined throughout the year for two years. Peaks in *N. battus* egg output were, in two consecutive years, determined in the February crop and the results of this group are presented below. As farm management aimed for a slow growing, lean, type of lamb, and securing a supply of meat for local markets throughout the year, lambs stayed on the farm for an unusually long time, allowing for a follow-up of the effects of both a spring and an autumn peak of larval

emergence in the same group of lambs. The group consisted of approximately 60 lambs for most of the season but towards the end of the year pairs of lambs were taken to slaughter each week, leaving a group of 40-plus lambs in December. Lambs were weighed at least monthly and, at the time pairs of lambs were taken away from the group for slaughter virtually all others had already reached their target weight. Therefore, the risk of introduction of sample bias towards smaller animals was deemed to be small. In both years there were one or two skinny animals showing stunted growth in the group and these were not sampled.

The group of lambs was circulated over 8-10 plots of pasture, being moved to the next plot every 2-3 weeks, in a random manner. Sampling commenced in April, when the lambs were approximately 8 weeks old and started to consume significant amounts of herbage. Approximately fortnightly, they were put onto concrete and observed. The first twenty freshly deposited dung samples were collected and processed individually. After lambs had been taken from the group the number of samples taken was adjusted so that at all times samples of one third of the group were taken. Many lambs in the group were slaughtered in the weeks before Christmas and at this time the study was aborted.

In the laboratory, egg numbers were quantified by a modified McMaster technique. 3 grams of faeces were added to 42 mls of tap water. Faeces were broken up into small pieces with a spatula and left to soak for one hour, after which the mixture was once again thoroughly homogenised with a spatula. It was poured over a tea strainer after which the filtrate was thoroughly mixed. 13 mls was immediately poured into a centrifuge tube and spun at 2000 rpm for 2 minutes. After the supernatant was carefully siphoned off a saturated NaCl solution was poured onto the pellet, up to the 13ml mark on the tube. The pellet was mixed with the salt solution and after thorough

homogenisation one chamber of a McMaster slide (Chalex Corporation, USA) was filled with a pipette. The centrifuge tube was once again homogenised, the other chamber filled, and the number of eggs within or on the lines of the two rosters counted. This number was multiplied by 50 to represent the number of eggs per gram of faeces. Remaining faeces were cultured at 25°C for one week, baermannised for 12 hours, and the L3-stage larvae present in the sample identified. Thus, the presence of *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* was established. *H. contortus* was, throughout the period measured, the dominant species by far, apart from during peak *N. battus* egg outputs.

Grenfell *et al.* (1987b) developed a model describing total faecal output of calves as a linear function of their bodyweight. This model did not prove to be directly transferable to sheep. From data presented by Anon (1993) it was determined that the dry matter food intake of growing sheep is also very well estimated as a linear function (see figure 7.9). Anon (1993) also showed that the weight gain of lambs remains relatively constant

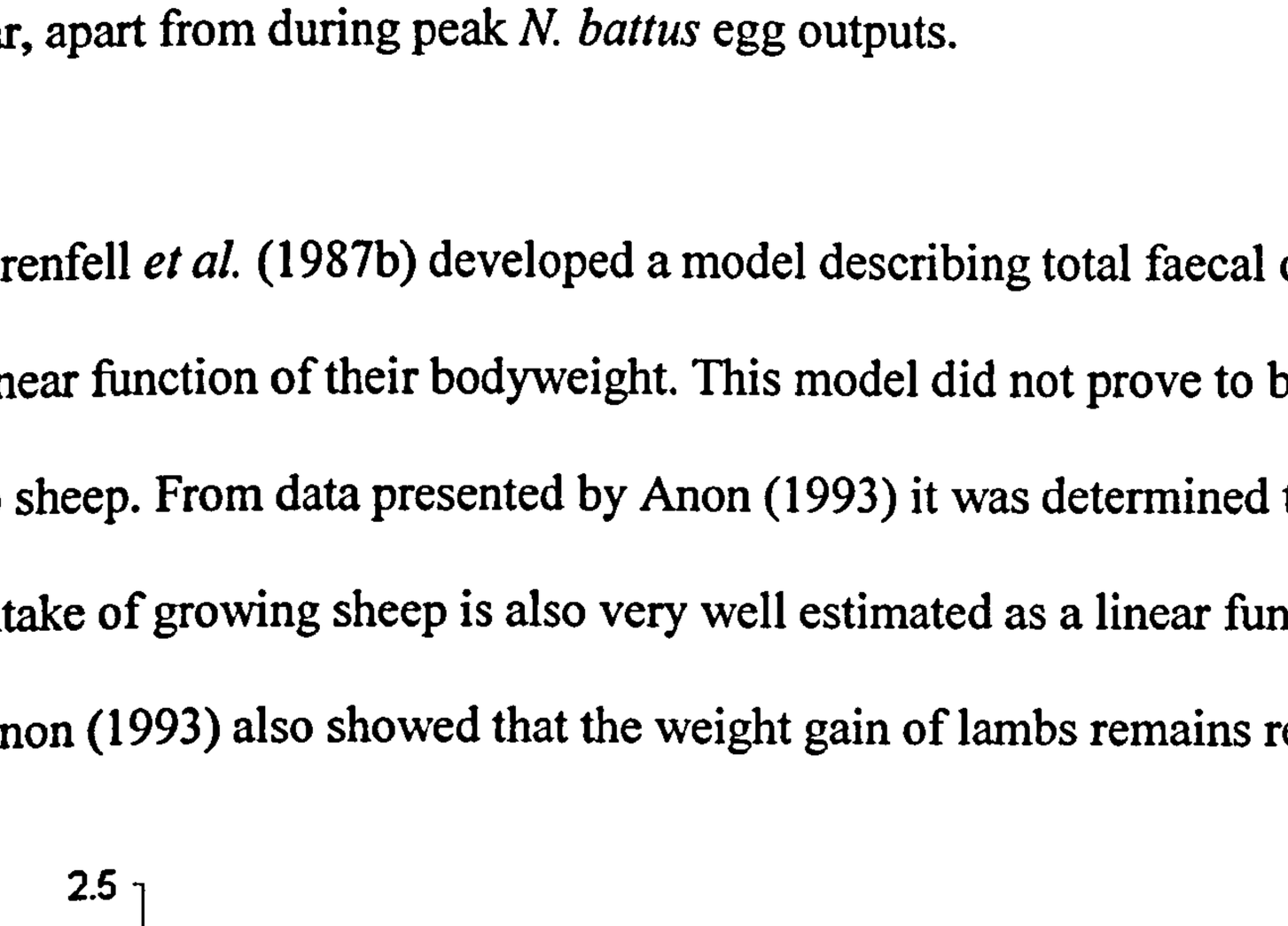


Figure 7.9 Dry matter intake of lambs as a function of their weight. Error bars represent 95% confidence intervals. Data from Anon (1993).

at 250-300 grams a day. It follows that faecal output of lambs is also likely to increase in a linear fashion. Thus the faecal output of 3-month old lambs was estimated at 300 grams (Thomas and Stevens, 1960) and the faecal output linearly increased with the slope of the regression line in figure 7.9, approximating the faecal output of a 35 kg lamb as 1 kg. The latter represents a realistic value for animals this weight (A.A. Donnan, personal communication). The bootstrapped mean group faecal egg count was multiplied by the mean faecal output (in grams) to give the mean number of eggs produced per lamb at the time of sampling. It was assumed that egg output took place at a constant rate over any 24-hour period. It was also assumed that faecal egg counts, between measured points, increased or decreased in a linear fashion and the mean estimated number of eggs per lamb per day was computed.

Soil surface temperatures were measured (as described in chapter 4) on the farm and the predicted days of maximum autumn hatch determined.

7.2.2 A stochastic model of *N. battus* offspring

A simple model, capturing the essentials of *N. battus* epidemiology only, was developed. This conceptual model predicts the 10-year offspring from a Markov-chain series of hatching events with, as parameters, the degree to which non-chilled eggs hatch and the relative success, in terms of offspring produced, of infections of immuno-naive lambs in spring and infections of animals with a degree if immunity in autumn, as follows. The model assumes that hosts are present when peaks occur and considers two hatching windows per year only. The probability distribution of completing the hatching process in the manner described above, for spring and autumn, in the Southwest and in Scotland, are derived from 10,000 stochastic hatching simulations of the hatching model

(Yeovilton/'Southwest': mean 0.70 (SD 0.37) in spring and 0.85 (SD 0.30) in autumn; Paisley/'Scotland': 0.97 (SD 0.34) in spring and 0.98 (SD 0.09) in autumn; probability of hatching ≤ 1). It is assumed that the number of offspring is a direct function of the probability of hatching. The number of offspring produced by any one hatch is modelled as a multiplication of the number of eggs available for hatching at that moment in time, the simulated proportion of eggs hatching (approximated by the probability of hatching), and a factor representing the number of offspring produced per hatching larva at the time of hatching (which is determined by host age/immunity). In each year, the number of eggs available for hatching in spring equals a proportion of the number of offspring produced in the previous spring, a proportion of offspring of the hatch in the autumn, 1.5 years earlier, and the number of eggs available for hatching in the most recent autumn but not able to complete the hatch at that time. The number of eggs available for hatching in autumn is a proportion of the offspring produced in the preceding spring, a proportion of the offspring produced in the most recent autumn and the number of eggs carrying over from the spring of the same year. The proportion of eggs hatching without chilling (i.e. both spring and autumn eggs in their first hatching opportunity) can simply be set as a value between 0 and 1. In year zero, the model is seeded with one embryonated egg. The total 10-year offspring produced (O_b) is the sum of the offspring produced in the ten springs and autumns. If eggs are assumed to live for up to 2 years then if $O_b < 5$ the parasite is predicted not to be able to persist. The model is not easily summarised in an equation but is illustrated in figure 7.10.

As a standard situation it was assumed that infections of spring-born lambs produce twice as many eggs per ingested larva than infections of immune animals in autumn (the 2:1 scenario). This may not represent the reality accurately but still makes a comparison of Scotland and the Southwest, as two UK 'extremes', possible. Four other scenarios

	Autumn (t-2)	Spring (t-1)	Autumn (t-1)	**Spring (t)**
HP				x* C + y*C + z
Ha				$HP*P_{s(t)}$
N			z	HP-Ha
O_b	x	y		$Ha*S_s$

Spring

	Autumn (t-1)	Spring (t-1)	**Autumn (t)**
HP			x*(1- C) + y*(1-C) + z
Ha			$HP*P_{a(t)}$
N		z	HP-Ha
O_b	x	y	$Ha*S_p$

Autumn

Figure 7.10 Predicted number of *N.battus* offspring at time (t) as a function of eggs produced in the previous year(s). t in years. HP = hatch potential, Ha = hatch, N = eggs not hatching, O_b = total offspring, C = proportion of eggs hatching after chilling only, Ps and Pa = stochastically modelled probabilities of eggs completing the hatching process in spring and autumn, respectively, S_s and S_p = offspring per L3 consumed (determined by the host) in spring and autumn, respectively.

were modelled:

(i) the 1.5-1.5 scenario, representing the same total success but with spring and autumn infections being equally effective in terms of the production of offspring, to test whether this, given the proportions of eggs that were found to hatch without chilling, is likely

(ii) the 2:0 scenario, testing the importance of spring-autumn interaction and representing a scenario where animals present on the farm in autumn do graze different pasture than that grazed by lambs in spring

(iii) the 0.5:1 , testing the effectiveness of an imperfect vaccine for lambs, reducing the offspring produced in spring by three quarters

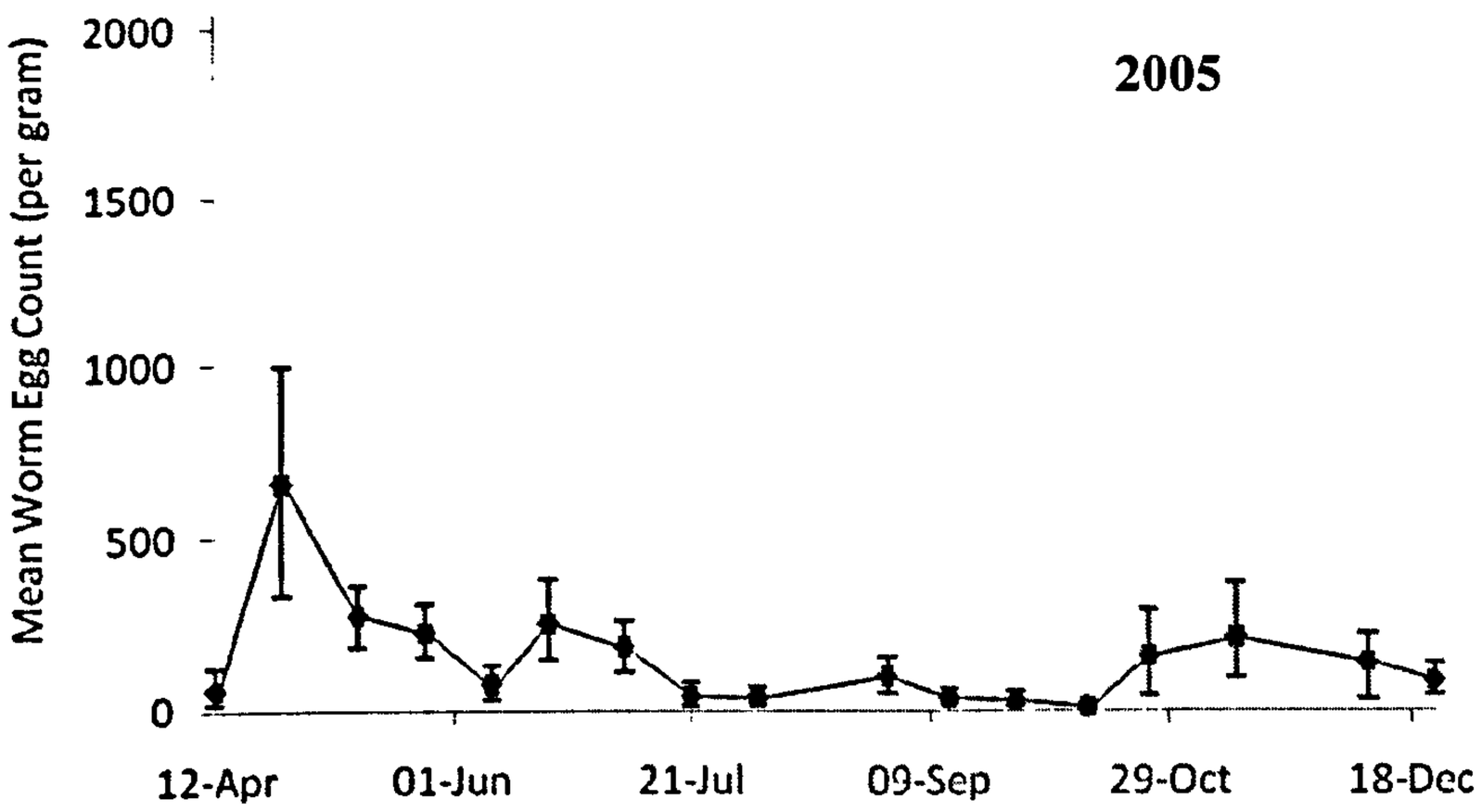
(iv) the 1:2 scenario, a situation where farm management, as a result of climate change, brings forward the lambing time to a point where young lambs start to pick up autumn-hatched larvae; At the time of the spring hatch the lambs are now already immune.

All five scenarios were simulated 10,000 times, for non-chilled hatch proportions of 0 to 1, increasing this proportion with increments of 0.1.

7.2.3 Results

Exploring contributions of autumn infections to total annual egg output

Bootstrapped mean egg counts are shown in figure 7.11. In both years a rapid increase in worm egg counts (WEC) was registered in April. This rapid increase was followed by a rapid decline, suggesting that in both years the immune system of the lambs was triggered to respond. In 2005, at the time of maximum egg output, a few lambs appeared somewhat listless while showing mild diarrhoea. This was also observed in 2006, when the measured spring peak in WEC was much more pronounced. In neither year did any of the lambs appear significantly ill in the period surrounding the maximum measured WEC. In 2005, WECs rose significantly for a second time, in June, suggesting a second peak of larval emergence. In the autumn, the 16th day of measured within-threshold temperatures was on October 15th in 2005 and September 21st in 2006. In both years these predicted maximum hatch dates were followed by increases in WECs but in 2005 in a more pronounced manner than in 2006. Corrected for total faecal output, the mean predicted daily egg output per lamb, and the cumulative egg output are given in figure 7.12. As expected, the egg output of lambs in spring was far greater in 2006 than in 2005.



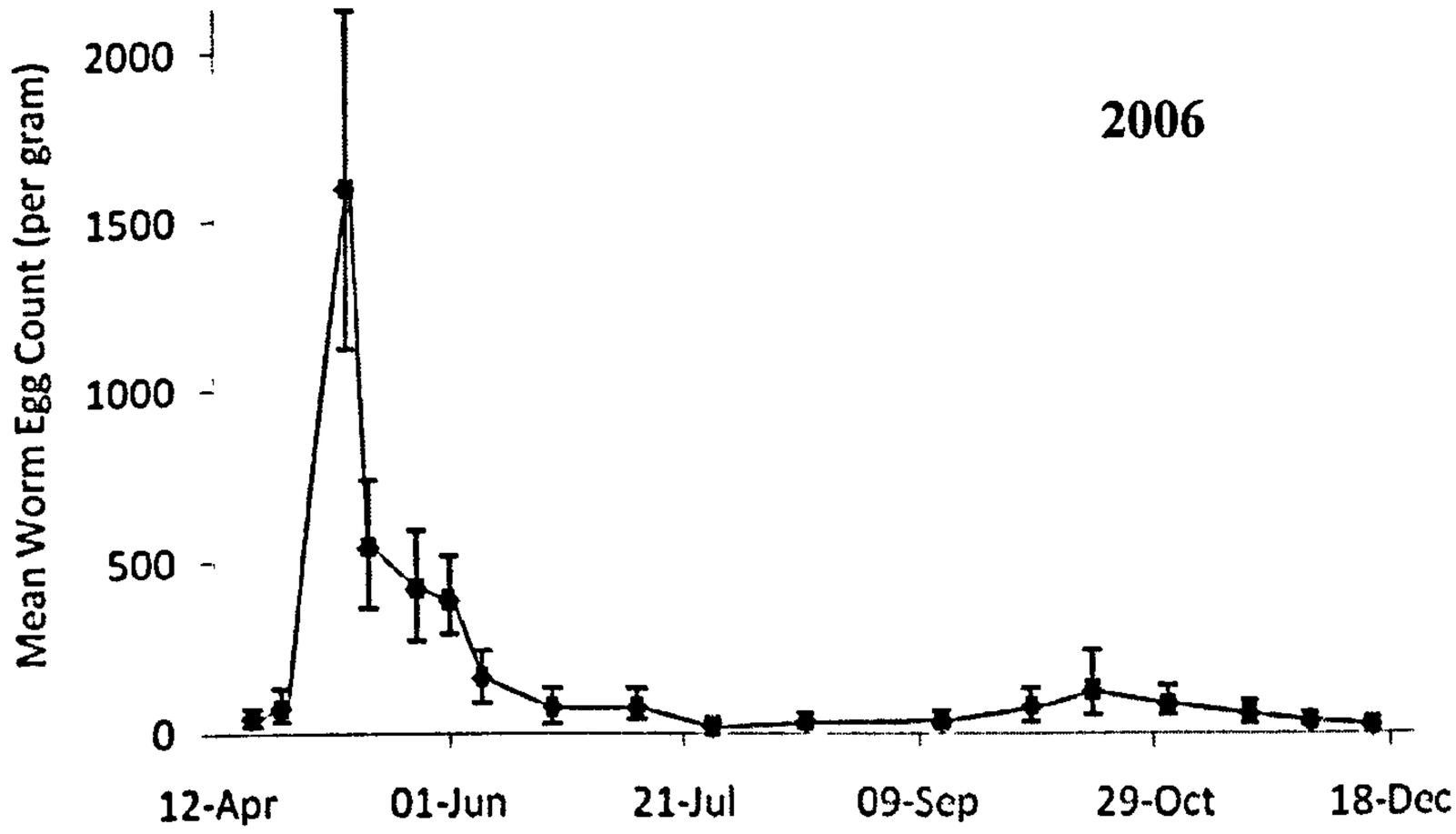


Fig. 7.11 Mean *N. battus* worm egg counts of groups of lambs, April-December, 2005 and 2006. Error bars represent the boostrapped 95% confidence intervals.

However, increases in egg output after suspected consecutive hatches, particularly the autumn hatch, were predicted to make up for the lower egg output in spring and, in autumn, 2005 total egg outputs were predicted to be higher than in 2006. Without following the autumn/winter egg production through, the ratio of predicted total spring (including summer): autumn eggs produced was 1.2 in 2005 and 1.8 in 2006.

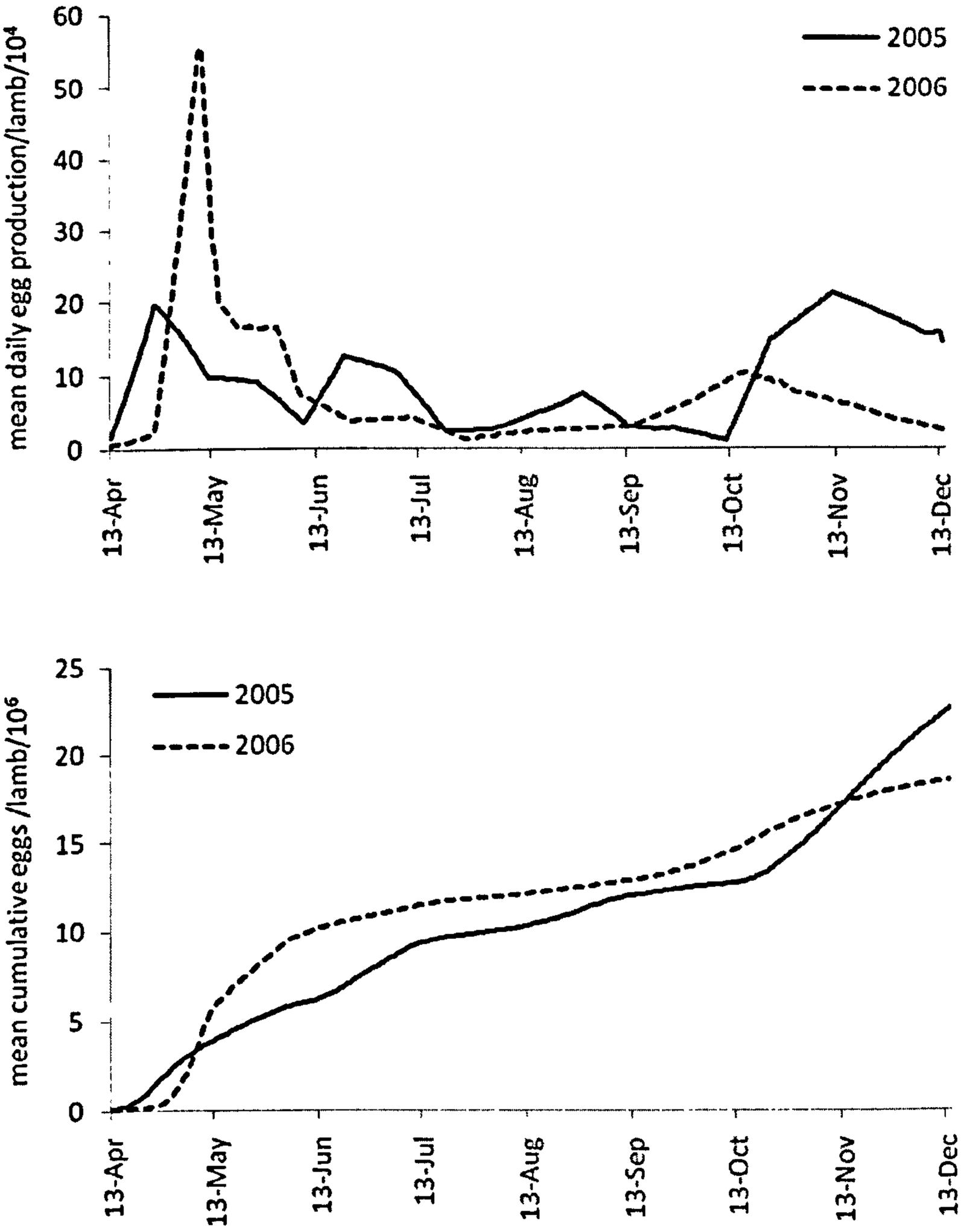


Figure 7.12 Estimated mean daily *N. battus* egg output, and the mean cumulative number of eggs produced, per lamb, 2005 and 2006.

A stochastic model of *N. battus* offspring

The 2:1, 1.5:1.5 and 2:0 scenarios are illustrated in figure 7.13. Regardless of the offspring per L3 scenario modelled, as the results of the higher hatching probabilities, the model predicts more offspring in Scotland. The 2:1 scenario predicts that it is

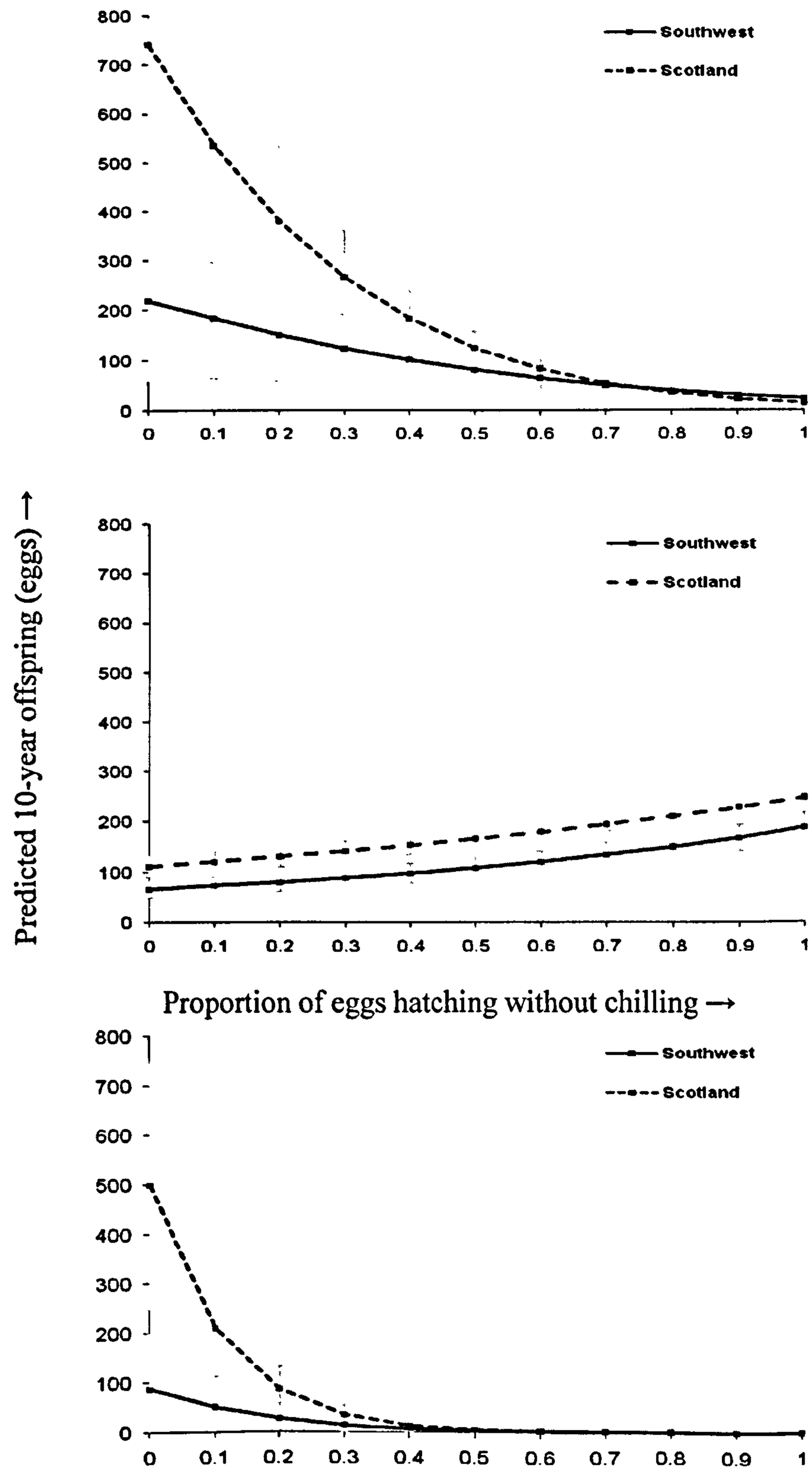
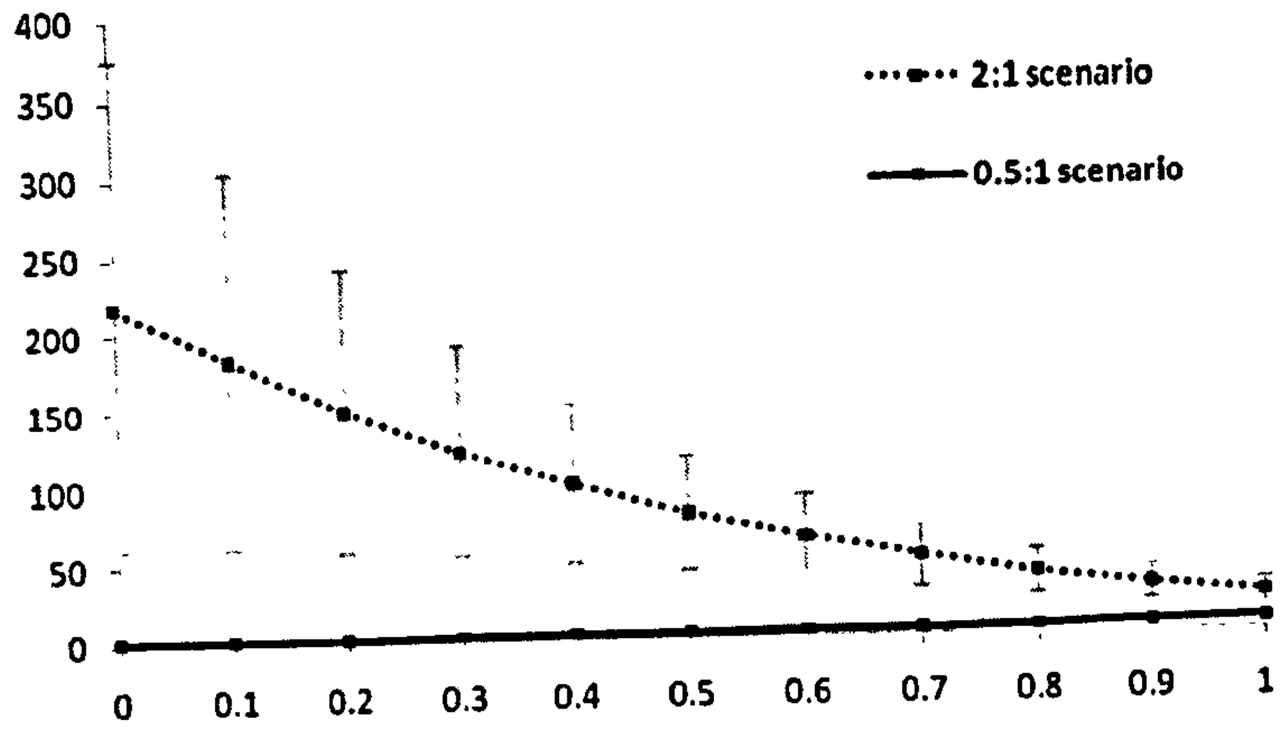


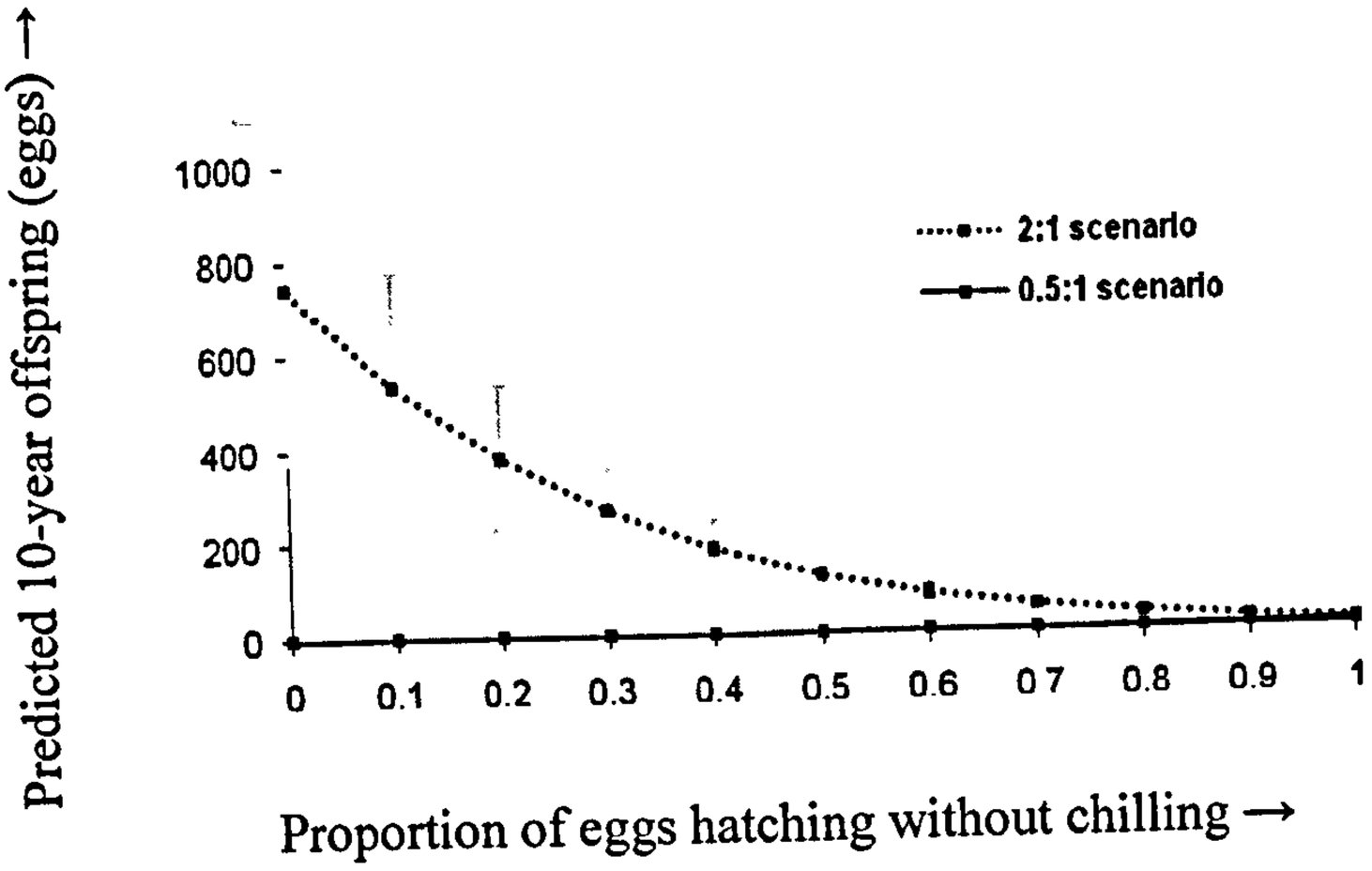
Figure 7.13 Projected *N. battus* offspring as a function of the proportion of eggs hatching without chilling in the Southwest and Scotland. The 2:1 scenario represents the situation where infections of young lambs produce twice as much offspring per ingested L3 than infections in immune animals; In the 1.5:1.5 scenario both lead to the same number of offspring; In the 2:0 scenario animals grazing in autumn do not contribute to the on-farm pool of eggs. Error bars represent 95% confidence intervals.

disadvantageous for eggs to hatch without chilling, whether in the Southwest or in Scotland. The confidence intervals of the two regions are very wide and overlap at every level of non-chill hatch. Only when spring and autumn infections lead to the same relative success in offspring (1.5:1.5 scenario) does it become advantageous for larger proportions of non-chilled eggs to hatch. If the relative success of autumn infections is increased further (1:2 scenario, figure 7.14) the projected number of offspring increases very dramatically with increasing proportions non-chill hatch. Reducing the egg output in spring (the 0.5:1 scenario, figure 7.14) is predicted to reduce the population to the point of non-persistence, regardless of the region. Non-sharing of pasture of autumn-grazing and spring-grazing animals is predicted to reduce offspring both in Scotland and the Southwest. At the proportions of eggs hatching without chilling encountered in the Scottish isolates (chapter 5) these would be predicted to persist even if the autumn pool of eggs is removed completely. The Bristol isolate (chapter 4) would be predicted to be eradicated from the farm at its high rate of hatching of non-chilled eggs. At lower proportions of non-chilled hatch the lower confidence intervals also touch the line of zero offspring.

Southwest 0.5:1



Scotland 0.5:1



Southwest 1:2

Scotland 1:2

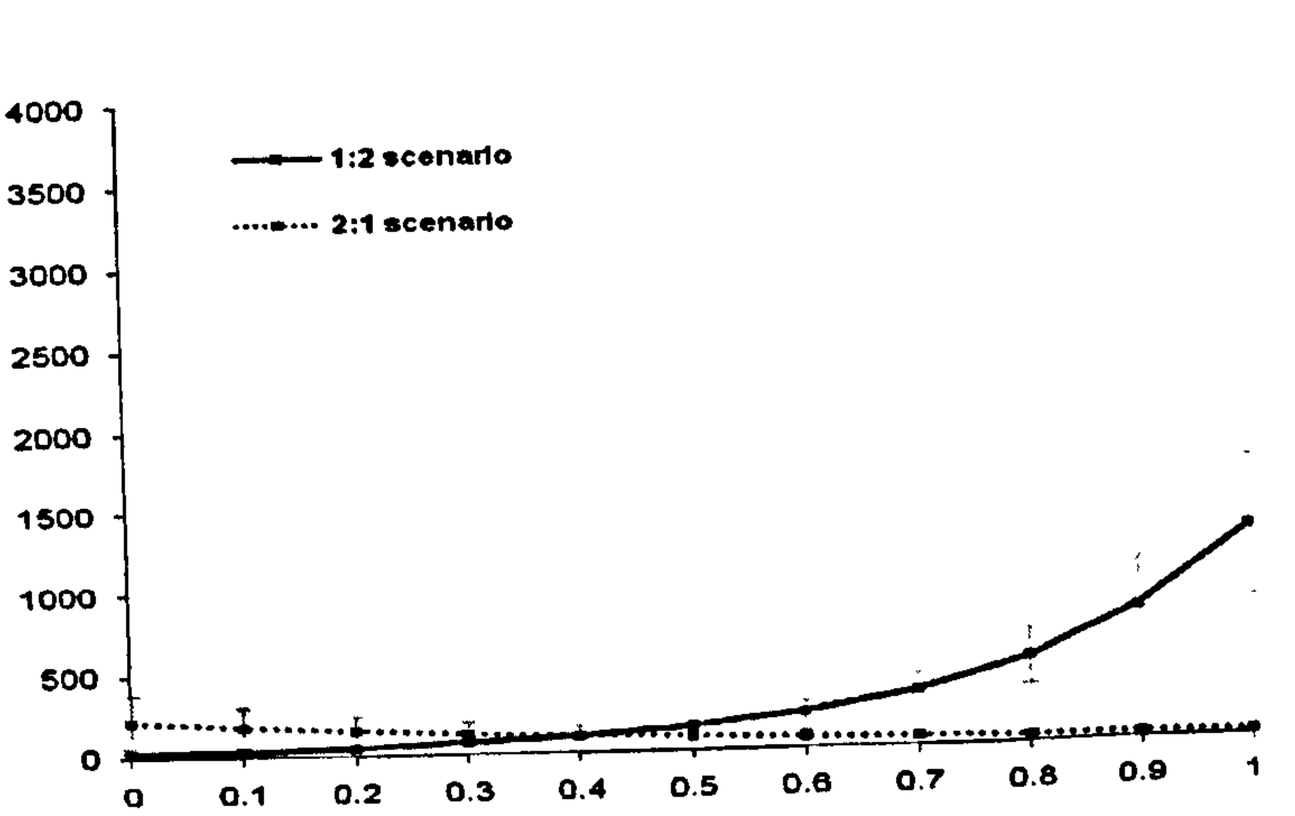


Figure 7.14 Projected *N. battus* offspring as a function of the number of eggs hatching without chilling and the success rate of spring (0.5:1 scenario) and autumn (1:2 scenario) infections. The 2:1 scenarios are given for comparison. Error bars represent 95% confidence intervals.

7.2.4 Discussion

Exploring contributions of autumn infections to total annual egg output

Lambs were circulated over pasture randomly and the level of pasture contamination was not measured in autumn. Therefore, as infection levels were in no way controlled, the 2005 and 2006 egg count results only represent two scenarios and it is not known whether they represent exception or rule. However, what is clear from the results is that autumn peaks of larval emergence may contribute very significantly to the total annual egg output. The rapid decline in WECs, and the mild clinical signs suggestive of *N. battus* infection observed in a few animals, suggest that infection levels in spring were above the threshold for immune response in both years. It seems therefore likely that the animals, in autumn, still had a degree of immunity and that a strategy of over-running the immune system of healthy animals with large numbers of infective larvae yields a considerable amount of offspring. Cornell *et al.* (2007) showed that the immunocompetence of rabbits dealing with *Trichostrongylus retortaeformis* infections wanes in the autumn and is also influenced by co-infections with other parasites. A phenomenon of waning immunity could explain the relatively large outputs of eggs in Autumn found here. Co-infections with other worms are, as both the weeks prior to the spring peaks and the weeks between the spring and autumn peaks were dominated by *Haemonchus contortus* infections, less likely to play a role. Other (micro) parasites were not tested for. High egg outputs in autumn may also be explained by the approximately 20-day time lag between recognition of infections with large numbers of larvae and the onset of worm expulsion (Lumley and Lee, 1981). This type of hyper sensitivity reaction is an all-or-nothing phenomena in which the endothelium of the villi of the affected part

of the small intestine are shed (Martin and Lee, 1980). If the number of incoming larvae exceeds that of the threshold for such an immune reaction (Conwill Jenkins and Phillipson, 1970; Rothwell, 1989; Medley, 2002) then, if the delay for the execution of such a reaction is a constant in non-immune and immune animals, infections of immune animals could be expected to be as successful as those of non-immune animals during the delay. Older, immune, animals would be expected to take up more infective larvae, in proportion to their higher dry matter intake, but do not normally show clinical signs of illness. This may be explained by aggregation of *N. battus* worms in the small intestine (Mapes and Coop, 1972): smaller animals would, relatively, shed a much larger part of their intestine.

The results also suggest that a strategy of lower infectivity in spring, possibly the results of the split peaks described above, can, in terms of annual numbers of offspring, be more than compensated for by higher infectivity rates in summer and autumn. However, maximum infectivity in spring is likely to shorten generation time considerably. A large proportion of 2006 spring-produced eggs is likely to have hatched in the autumn of the same year. Most 2005-produced eggs will only have hatched either one or one and a half years later.

On this farm a relatively large number of lambs remained on the farm in autumn. Some farms may graze store lambs throughout the autumn and winter and on these farms pasture contamination during these months may indeed reflect levels described here. On many other farms only replacement ewelambs (up to 25% of the lamb crop) and ewes will remain on the farm in autumn. Thomas (1959b) showed that approximately 40% of ewes may excrete 50 eggs per gram of dung and their contribution to pasture contamination might have been underestimated. The exact relative success of spring and

autumn hatches in terms of contributions to annual offspring, is therefore hard to estimate. The present study suggests, on the farm under study, ratios varying between approximately 2:1 and 1:1. It is not known whether infection levels may have been higher in autumn than in spring.

This study highlights that, although disease is not as often diagnosed in the Southwest, the parasite may still be thriving. Also, the potential for serious disease remains and it may strike highly unpredictably, noticeably if several years of '2005 epidemiology' are followed by one similar to 2006, in which spring hatching circumstances were very good. Given the extensive grazing strategy and frequent pasture rotations on the farm it is remarkable that, apparently, all spring and autumn peaks of larval emergence were recorded. All occurred on different plots of pasture and this may show the importance of an autumn hatch, complementary to the spring hatch, and perhaps the egg output of 'low-responders' throughout the summer, for the persistence of the parasite on farms with unpredictable grazing management.

A stochastic model of N. battus offspring

The model predicted greater success of *N.battus* in Scotland in all scenarios where the parasite population was predicted to persist. Another consistent, but more surprising, finding was that trends for each scenario were similar in both regions. The strongest dependence on a spring hatch was associated with the greatest uncertainty (largest standard deviations) in numbers of offspring in both regions. It is remarkable that this 2:1 scenario, at the given probabilities of hatching in the two regions, predicted lower proportions of eggs hatching without chilling are beneficial to parasite populations in the

Southwest. The 1.5:1.5 scenario showed only moderate increases in predicted offspring towards the higher end of the non-chill hatching scale and this may reflect a decrease in generation time. Only when the offspring per larvae was set as twice as high in autumn as in spring did it become dramatically advantageous to increase the proportion of eggs hatching without chilling. It is not known which one of these simple scenarios reflects reality best. However, the results clearly show that the predicted success of 'hedging bets' is very sensitive to the relative success of autumn infections. If the contribution of autumn infections to the pool of eggs at pasture increases (1:2 scenario), bet-hedging becomes a must for persistence of the population.

Assuming that the Bristol isolate (chapter 4) represents the Southwest better than any of the three Scottish isolates (chapter 5) the question arises how these findings can be interpreted against a background of predicted climatic pressure on *N. battus* to increase proportions of non-chilled eggs hatching. The only available hypothesis appears to be that infections of immune animals must be more successful in the Southwest than in Scotland. It may be climatic pressure, and the subsequent shift in the proportions of non-chilled eggs hatching, itself that makes the autumn infections more successful. As numbers of larvae present in autumn increase the infections take on the character of mass-invasion, triggering a hyper-sensitivity/worm expulsion type of reaction, rather than trickle infections. Regardless of the mode of infection, post immune response parasite burdens are normally reduced to, and subsequently kept at, a certain threshold level (Conwill Jenkins and Phillipson, 1970; Behnke *et al.* 1992; Medley, 2002; Rubio-Godoy and Tinsley, 2002). In situations with high host-densities but with short-lived hosts, or hosts regularly dosed with anthelmintics, a period where mass infection over-runs the immune system is, during the delay for the hypersensitivity response to take effect, likely to have advantages to the parasite over a trickle-type infection. A

preliminary model of *N. battus* host immunity indeed showed mass infections, up to the point of host death, to be highly beneficial to the parasite (van Dijk, J., unpublished data). In this way, climate-driven increased proportions of non-chilled larvae hatching could, once autumn peaks went over a threshold for a hypersensitivity-type reaction by the host, be reinforced by increased egg outputs in autumn. Thus it would seem likely that high levels of non-chill hatch are required for autumn infections to become beneficial. Therefore it is unlikely that an increase in proportions of eggs hatching without chilling was, initially, driven by host immunity.

The predicted dramatic increase in offspring for *N.battus* larvae infecting lambs in spring/winter is rather worrying. As the result of climate change, and an earlier start to seasonal grass growth, farmers may strive for the lambing period to be brought forward. In the Netherlands, an increase in the use of breeds able to produce 3 lamb crops in 2 years has already been witnessed (Eysker, M., personal communication). Such management changes may lead to dramatic changes in parasite epidemiology, and the timing of disease. On the far north coast of Scotland these type of infections may already be a reality. The area around Thurso, on the far north coast of Scotland, is heavily influenced by the warm North Atlantic gulf stream and temperatures are very similar to those experienced in the Southwest. In recent years, farms lambing towards the end of the year regularly suffer heavy *N. battus* infections in January or February, long before a spring hatch would be predicted to take place (Clark, A., personal communication).

As there is a high degree of temporal and spatial uncertainty in the occurrence of spring and autumn peaks *N. battus* infections are not easily controlled by the use of anthelmintics. The model shows a dramatic fall in predicted total offspring both when the

number of eggs deposited in spring and when eggs deposited in autumn is reduced and this suggests a strong interaction between spring and autumn peaks. Assuming a 10-year compliance of farmers with vaccination campaigns, the use of an imperfect vaccine is predicted to be highly successful. A vaccine is currently not commercially available. Apart from not grazing pasture grazed by lambs in the previous spring, non-grazing by lambs of pasture grazed by stock in autumn and winter would be predicted to be a highly successful strategy. Especially in the Southwest, this prevention of interaction of autumn infections with spring infections would be predicted to yield great success. However, unless ewelambs and ewes are grazed away from the farm in autumn/winter, logistically, it will be difficult to achieve this while fitting in with grazing strategies designed for the control of other gastro-intestinal nematodes.

7.3 Conclusions

The modelling of *N.battus* ecology is possible making use of simple models. The developmental phase is predictable and its modelling can be accomplished by taking into account a few simple rules. However, as hatching peaks consist of pools of eggs with a very different history, the modelling of the magnitude of a peak of larval emergence is not easily accomplished by traditional models of gastrointestinal nematode epidemiology. Conceptual probability based models may be better placed to investigate the interaction between spring and autumn peaks.

Climate change may increase the number of eggs produced after the peak egg production, by 'low-responders', able to contribute to the following spring peak. However, the main effect of climate change on *N. battus* ecology is likely to be a decrease in the probability

of hatching in spring. Therefore, the epidemiology of the parasite in Scotland is indeed predicted to become more like that observed in the Southwest. It is likely that fewer years with high disease incidence will be witnessed but, crucially, the potential for serious disease outbreaks will remain while their timing will become more unpredictable. Another potential threat to animal welfare is the potential for early-born lambs to pick up autumn infections. If these type of infections became commonplace very serious disease outbreaks would be predicted.

Adaptation of the upper threshold for hatching is unlikely to take place within several decennia. One way of parasite adaptation to climate change seems to involve the hatching of non-chilled eggs. As a result, older animals will encounter higher levels of infection. The immune reaction of such animals to these infections is an area in urgent need of research attention. Initial results indicate that their contribution to total on-farm egg output is highly underestimated.

The control of the parasite on the farm is very much complicated by the importance of autumn infections in the epidemiology. On most UK farms it will be difficult to control the parasite by grazing management. A vaccine was predicted to be highly successful but is currently not available.

Chapter 8 - Concluding discussion

The thesis has documented the effects of temperature and rainfall on the development, hatching, survival and migration of the free-living stages of *Nematodirus battus*, and *N. filicollis*, in detail for the first time. It also described essential temperature-influenced dynamics of free-living stages of *Teladorsagia circumcincta*, *Trichostrongylus colubriformis* and *Haemonchus contortus*. The findings have been discussed at length in the previous chapters and will not be repeated here. This chapter wants to integrate findings across chapters, put them in the context of the aims and objectives defined at the start of the study (chapter 1), and prioritise future research.

8.1 Past, present and future

The study of the effects of climate change on the epidemiology of infectious diseases is only at its beginning stages and needs to gather momentum urgently. Recent studies have focussed on the increased risk of transmission of exotic (vector borne) viruses and parasites in the temperate regions and endemic pathogens have been ignored. This study has provided evidence that, as a result of global warming, the epidemiology of highly pathogenic parasites of sheep is already changing and that this is likely to be a continuing process. The exposure of vector-borne parasites to environmental temperature is buffered by the vector and under influence of vector behaviour. The nematodes studied here are in direct contact with, and strongly dependent on, their environment for prolonged periods of time and therefore provide very sensitive models for the study of the effects of climate change on infectious pathogens. Moreover, the different species have very different over-wintering strategies

while the degree to which the host is utilised for over-winter survival appears to be both highly adaptable and influenced by climatic factors. Changes in the timing of, and the proportion of populations undergoing, hypobiosis may be early indicators of responses of many living organisms to extended developmental windows in autumn, spring and winter.

The study of effects of climate change on infectious parasites could be in danger of following the trends in meteorological research and focus on simulation models predicting the future. In particular in the temperate regions it may be fruitful to see the present as a continuously shifting crossroads of pathways from the past to the future and from the Arctic to the Tropics. What has happened before is likely to happen again.

At this moment in time climate change appears to already affect parasite ecology significantly, and therefore the study of the recent past is important. The analysis of large datasets on parasite abundance, spanning many years, is therefore likely to be a method yielding results (Poulin, 2007). However, such databases are very scarce and they limit the number of species that can be studied. The epidemiology of gastrointestinal nematodes appears to change rapidly as the result of small increases in temperature. Where databases do not exist it is important to intensify the documentation of the current expressions of parasite traits, and parasite abundance. For databases documenting parasite abundance it is vital to centrally agree protocols in use in contributing laboratories.

The study of likely adaptations of parasites to climate change should exploit north-south differences. Arctic parasites persisting in the temperate regions are models for adaptation to

warmer climates, while tropic-adapted parasites may reveal likely future adaptations. Moreover, significant north-south differences in parasite epidemiology were detected even on a relatively small island like Great Britain. Differences in environmental temperature, although small, are likely drivers behind the disease patterns uncovered. This study also showed that measurable differences in parasite adaptation are likely to exist at different geographical locations in the UK. Making use of translocation experiments, this opens the door for a realistic study of adaptation rates.

It is encouraging to see that very simple stochastic mathematical models, parameterised from laboratory experiments and validated with pasture experiments, appear to capture the seasonal abundance of infective larvae very well. Two simple temperature driven models provided explanations for every single disease trend uncovered at the start of the study. Such models are easy to handle and analyse and are excellently placed to investigate general future trends while providing a confidence interval for these trends. Sensitivity analyses of these models can also give very valuable information on likely future adaptations. Given the rate at which changes appear to take place, the integrated use of computer models is likely to be essential in the design of future worm control methods. Pasture studies investigating the use of novel control methods are normally tremendously laborious and have to span several years, making them very costly (see, for example, Eysker *et al.* 2005a). Also, their results strongly depend on between-year climate variability (Eysker *et al.* 2005b) and will therefore, per definition, have to be extrapolated to other years. To my knowledge, in the field of Veterinary Parasitology, computer models are rarely used at the design-stage of such studies and much would be gained from informed study design. At the analysis stage of such pasture

studies, models could inform investigators on the probability of the findings happening in other years. Apart from climate stochasticity, parasite adaptation could be explored and worst-case, and best-case, scenarios modelled.

A next step in the modelling of the effect of climate (change) on parasite abundance is the stochastic incorporation of realistic between-day, and inter-annual, climatic variability and climate change scenarios in the models. This task can only be undertaken in close collaboration with climatologists. A more purely parasitological challenge ahead is the realistic stochastic modelling of egg outputs of sheep of different ages, with differences in current and previous worm burdens and larval ingestion rates. The logistics of models simultaneously tracking parasite abundance on several pastures, linking it with grazing history, also present a challenge. Morgan *et al.* (2006), working on parasite exchange between groups of (migrating) hosts, have shown that this is achievable.

8.2 Key determinants of parasite abundance

At the time steps of years, months and days modelled in this thesis, all research questions could be answered studying the effects of temperature on the free-living stages. Temperature appears to be the single most important climatic factor influencing parasite abundance.

In the UK, rainfall was found to be an unlikely limitation to the development, hatching and migration of larvae onto herbage. Water may well be limiting the migration of infective larvae out of dung. While studying the possible effects of rainfall on faecal degradation, using 30-year rainfall data of three weather stations, is became clear that fortnights without

rain are rare even during summer but this may change in the future (Hennessy *et al.* 1997). Therefore, losses of L3 in dung may increase but also the risk of disease outbreaks immediately after spells of rain. However, it is currently not clear whether the moisture provided by dew is sufficient for larval migration out of dung.

The eggs of Nematodirae can also be trapped in dung and this appears to damage the eggs for reasons other than desiccation. Disintegration rates of eggs in dung are currently not known. Losses of the pre-infective stages of *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus* species the result of lowered relative humidities have been researched extensively in the laboratory. However, it is currently not possible to translate this work to pasture reality.

Under UK farming conditions of high host density I propose that temperature thresholds are the key climatic determinants of parasite epidemiology. For all species apart from *Nematodirus battus* the minimum threshold for development appears to be most important determinant of patterns in parasite abundance. Differences in this threshold as small as 1°C were predicted to have important consequences for parasite success, and perhaps over-winter strategies. For *N. battus* the temperature thresholds between which a hatching stimulus is experienced are crucial. The mimimum egg development threshold of *N. battus* prevents hatching of autumn eggs in the following spring and is therefore also likely to be an important co-determinant of parasite epidemiology in the UK.

A second very important epidemiology-determining parasite characteristic is its propensity to increase the proportions of eggs developing, and development rates, at increasing above-threshold temperatures. Published studies have put much emphasis on the optimum

development temperature of parasites. However, parasite abundance is also very much determined by development rates around the minimum development threshold. For Arctic parasites like *N. battus* and *T. circumcincta* these rates appear to be relatively close to the rates at optimum development temperature and for *T. circumcincta* this leads to a relatively flat curve of parasite abundance. The slope of the increase in the proportion of eggs benefiting from increasing temperatures is steepest for *Haemonchus contortus* and this is likely to co-determine its 'peaky' abundance at pasture. How well parasites are able to apply extra thermal energy between the minimum development threshold and the optimum development temperature, and how steeply egg developmental success declines at temperatures over the optimum temperature, is also likely to determine whether they will benefit from global warming. If the slope of developmental success up to the optimum temperature is steep, and the optimum temperature is relatively high, increased mean temperatures may result in an increase in parasite abundance.

Developmental success rates are likely to be stronger determinants of parasite abundance, and disease, than larval death rates. However, within the window of opportunity for larval development, larval death rates determined the timing of peak abundance and the accuracy of future trend predictions. Therefore, the study of drivers of larval death cannot be ignored. A recurrent theme in several chapters was that temperature cannot explain larval death rates observed at pasture, and neither can desiccation. If we do not know why larvae die faster at pasture than in the laboratory we can also not determine larval death rates under changed climatic conditions. Sunlight is, apart from temperature and desiccation, the only suggested cause of larval death at pasture (Tromba, 1978).

8.3 Future changes

The study of the short-term effects of climate change on the free-living stages of parasites is only a starting point and is of little value without explicitly studying effects on hosts and on parasite evolution. Veterinary parasitologists traditionally have a strong track record in work on the free-living stages, and dose-response studies in hosts, but not in evolutionary biology and greater collaboration with biologists will be required.

Temperature thresholds appear to be key determinants of parasite epidemiology yet there is not much evidence that they adapt when environmental pressure is applied.

One the one hand this may just show that lowering a development threshold holds little advantages. It could be argued that such advantages will be greatest when the proportion of the population able to develop at the minimum development threshold temperature is high. This would make *Teladorsagia circumcincta* the most likely species to adapt this way. However, a relatively large number of larvae of this species over-winters at pasture and these are offspring of worms that normally have developed during the summer. Also, in winter development rates will be low and by the time development rates increase rapidly (during the summer) all other eggs will also be able to develop. Worms with a lower development threshold may therefore remain a small minority of the total worm population. Troell *et al.* (2005, 2006), comparing Swedish and Kenyan *Haemonchus contortus* isolates, found no evidence of adaptation of development and survival characteristics of the species to the Swedish environment. However, it has to be remembered that the species, in Sweden, entirely relies on hypobiosis for over-winter survival (Waller *et al.* 2004). Therefore, if the

development of arrested larvae is completed at a time when temperatures have risen above the development threshold, no selective pressure would be applied to adapt the development threshold. Selective pressure may only be strong if environmental temperatures are around the minimum development threshold during the summer. Still, Crofton *et al.* (1965) compared the minimum development thresholds of three *H. contortus* isolates, one from the UK and two from the USA, in the laboratory and found the threshold of UK isolates to be as much as 6°C lower. Crofton *et al.* (1965) estimated that such a divergence would have occurred over a time frame of at least 50-60 years.

Nematodirus battus was first described in the UK approximately 60 years ago but it is unlikely that its upper temperature threshold for hatching has adapted rapidly during those years. The model predicted that the infection of lambs in spring still constitutes the best strategy for the parasite and the significantly higher incidence of disease in Scotland is likely to reflect this. In the Southwest, the model also showed that a 1°C rise in temperature would improve the probability of eggs hatching more than very high proportions of eggs hatching without chilling. The high proportion of eggs hatching without chilling on a farm in the Southwest therefore suggest that the threshold for hatching is not as easily adaptable as a change in the proportion of eggs hatching without chilling. The adaptability of this hatching behaviour was also shown by the 100% chilled hatch of *Nematodirus filicollis* isolated on the same farm.

I conclude that temperature thresholds determining the epidemiology of parasites may be adaptable to new environments but at lower rates than other traits determining the timing of the appearance of infective larvae at pasture. In order to predict short to medium long-term changes in the epidemiology of parasites I therefore propose to focus future research efforts

on these traits. This study identified hypobiosis, and the hatching behaviour of Nematodirae, as likely rapidly adaptable traits.

Phenotypic plasticity in the hatching behaviour of *Nematodirus battus* was suggested by Thomas (1991). Although this study did not confirm that the proportion of *Nematodirus* eggs hatching without chilling is a plastic trait all findings suggest that it could be. If the trait is indeed plastic *Nematodirus battus* could prove an exciting model for the study of rates of adaptation to climate change. One future research priority is therefore to determine whether the trait is plastic and at what rates changes in hatching behaviour occur.

Currently, there are many gaps in our understanding of the phenomenon of hypobiosis. We do not know exactly what it is targeted towards and know little about how cues affect the behaviour. From studies conducted in Africa it would be predicted that lower proportions of larvae will hypobiose if developmental windows at pasture expand. However, it is unclear what exactly would drive such changes.

Gaba and Gourbiere (2007) showed that the duration of hypobiosis is crucial to the success and stability of parasite populations. This may suggest that the influence of host immunology and environmental cues have been over estimated. A certain proportion of the larval population may arrest year-round and high numbers of larvae arresting development in autumn may be artefacts of the high infection rates toward the end of summer. The work by Gaba and Gourbiere (2007) also shows that, if the timing of the arrest of development were to change as a result of climate change, the consequences for parasite populations are highly unpredictable. The study of hypobiosis is also of importance because the trait directly affects

options for future control. If proportions of larvae arresting increase our reliance on anthelmintics will increase. A strategy of virtually all parasites over wintering in the host make the population vulnerable to extinction when the hosts are dosed with anthelmintics but, paradoxally, such a strategy also enhances the rate of development of anthemintic resistance. This poses serious challenges to the control of *Haemonchus contortus* on UK farms.

All species are predicted to expand autumn transmission windows and, through an increase in the number of doses given, this is likely to further increase the development of anthelmintic resistance. It is also likely to lead to increased contributions of mature animals to parasite offspring, in autumn. Higher proportions of parasite populations may hypobiose in adult animals leading to higher burdens of infective larvae at pasture early in the following year. The contributions of older animals to the total egg output at pasture are currently unclear and urgently need investigation. This thesis has repeatedly suggested that the immune system may buffer increases in the force of infection far from completely.

Especially for the species *Nematodirus battus*, an increased importance of the role of adult animals in parasite epidemiology would poses serious challenges to the control of these parasites by grazing management. The option of separation of pastures grazed by adult animals and lambs has to be investigated further.

In summary, I identify the following priority areas for further research:

1. quantifying contributions of immune animals to parasite populations

2. the study of directions and rates of parasite adaptation: phenotypic plasticity in *Nematodirus*, and hypobiosis in *Haemonchus contortus*

3. the modelling of the use of grazing management as a tool for control of gastrointestinal worms under changed climatic conditions

4. increasing our understanding of climatic determinants of larval death rates at pasture.

Appendix – Transforming air temperature into soil surface temperature

Introduction

Levine and Todd (1975) showed that air temperature is a poor estimate of the soil temperature experienced by trichostrongylids. Field stations contributing to the historic database of the MET-office have recorded some scanty data on soil temperatures taken at 30 cm depth. From these data a 30-year temperature distribution cannot be constructed while the temperature at this depth is also unlikely to represent soil surface temperatures adequately. All stations have traditionally recorded air temperature at 2 meters above the soil surface. For modelling purposes, the question therefore arose how to estimate soil surface temperature (SST) from air temperature (AT).

The temperature in the top layer of the soil is under influence of the air above it but also buffered by the soil layers underneath it (deeper soil temperatures, DST). It would therefore seem likely that differences between SST and AT increase with increasing discrepancies between AT and DST. This would mean that the difference between SST and AT is not constant over the seasons. Also, the discrepancy between AT and SST may be smaller for daily minimum temperatures than for daily mean and maximum temperatures. Apart from the soil underneath it, SST is also likely to be buffered by the biomass of the herbage above it. Both heavy rainfall and evaporation of water after a spell of rain may rapidly lower SST, and may lower SST more than AT. Rainfall would then be most likely to influence the difference between AT and SST most during the warmest months of the year, and be a stronger influence on the daily mean and maximum temperatures than on minimum

temperatures. Lastly, as air temperature data from both the Southwest of England and Scotland were used for the modelling and mean, maximum and minimum air temperatures of these regions are likely to differ in magnitude all year round, it is unlikely that one simple correction factor can suffice for both regions.
It appears a framework for converting AT into SST, applicable to all regions, is needed.

Materials and Methods

In December 2004, a Skye MiniMet weather station (Skye Instruments Ltd., UK) was installed at Lower Failand Farm (near Bristol), the site also used for the sampling of herbage and dung described in chapters 4 and 7. This weather station had been calibrated by Skye Instruments Ltd., UK, before use. The air temperature-measuring probe of the station was installed at 2 meters above soil level and the external, soil, probe in the upper 1.5 cm of soil, between the grass roots. The respective temperatures were logged every 15 minutes. The daily number of millimeters rainfall were also measured and recorded by the weather station. At the start of 2006 the weather station broke down and had to be replaced. Two new, freshly calibrated, TinytagPlus® temperature loggers (Gemini Data Loggers, UK), again logging temperature every 15 minutes, were installed in the manner described above and kept running throughout 2006 and 2007. Before the installation of the loggers, and at the end of 2006, the external probes of both loggers was put in approximately 500 mls of well-mixed, lukewarm, water and left to stand at room temperature for 5 hours while the temperature was measured every 15 minutes. The temperature measurements of the loggers

were compared in a Chi-square test and no significant differences were detected in the measurements ($X^2_{(19)} \leq 3.3$, $p \geq 0.99$).

Biweekly, the grass length of the herbage around the probes was inspected and, if necessary, clipped back to a length of approximately 15 cm.

From the measurements the daily mean temperature, maximum temperature and minimum temperature were computed. The data gathered for the year 2005 was analysed in detail while the data gathered in subsequent years was used to scan for inter-annual differences. For every day of the year, the mean, maximum and minimum air values were deducted from their respective soil values. In order to find out whether a simple year-round correction factor could be applied the resulting values (the difference with air temperature, $D_{AT,}$ i.e. a value to be added to air temperature to get the estimated corresponding soil temperature value) were analysed in a one-way ANOVA and Tukey's pairwise comparison, with as factor month of the year. As February had 28 days only the first 28 days of each month were included in the ANOVA. Following this, the D_{AT} values of clusters of months identified as not significantly different in the ANOVA were linearly regressed on AT.

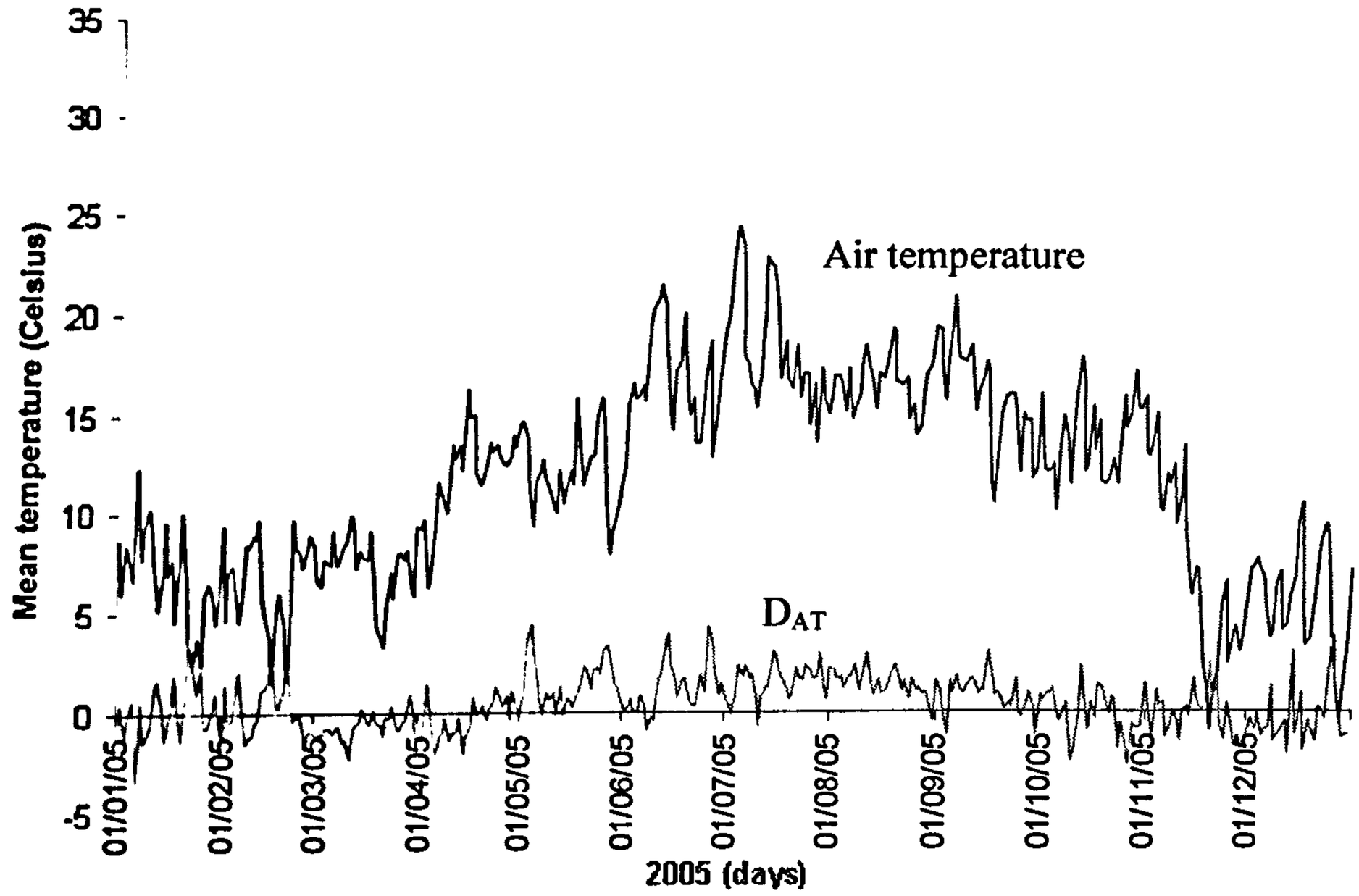
For the warmest months of the year, May to September, the influence of adding the factor rainfall to the predictive model was tested in an analysis of covariance (ANCOVA).

Results

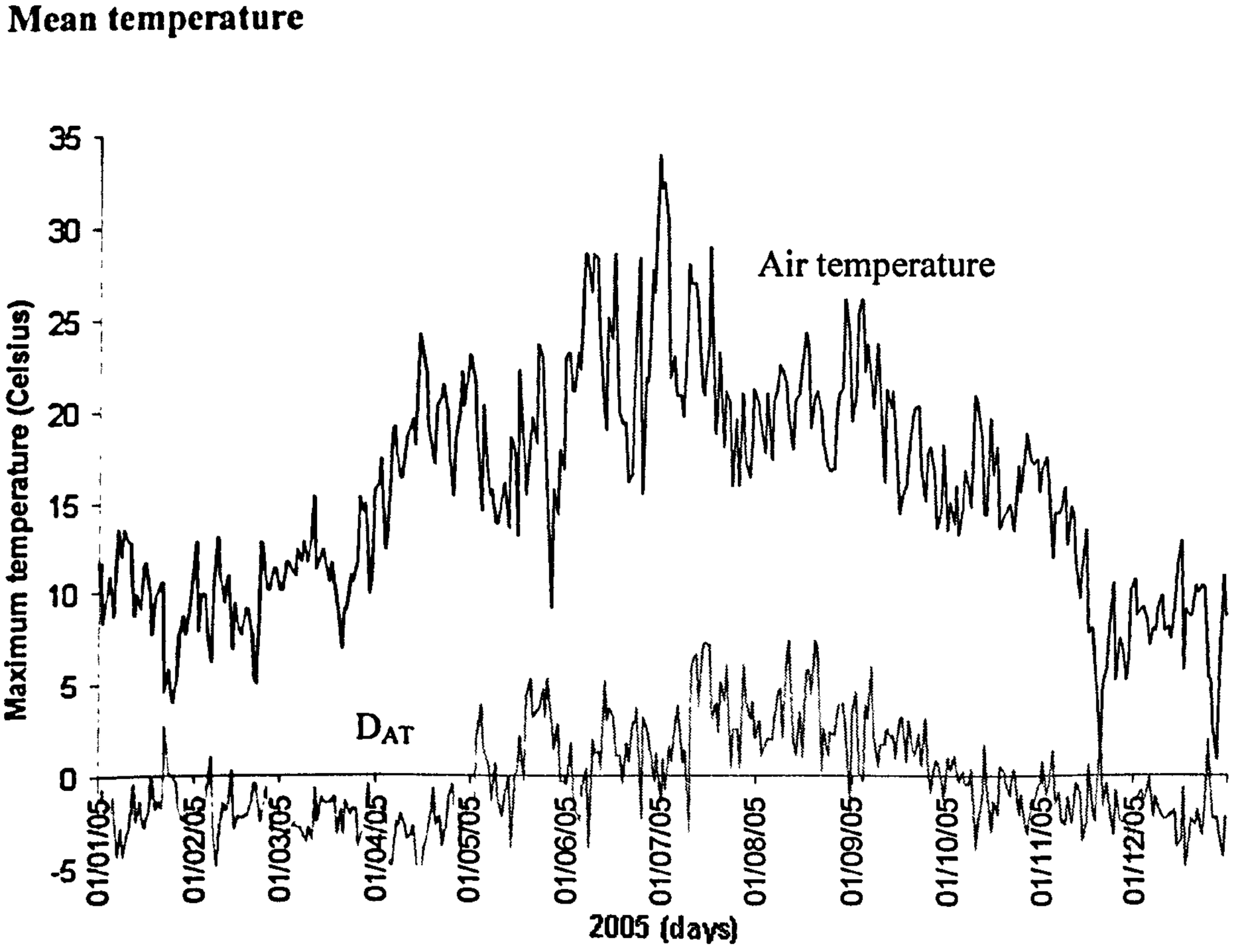
Mean temperature

The mean AT measurements, and the corresponding difference between mean soil and air temperature, are given in figure 1A. In the Southwest, during the coldest months of the year, $D_{AT\text{-}mean}$ values are small but the mean air temperature is, on average, somewhat higher than the mean soil temperature. However, as the soil warms up as the result of increasing air temperatures, approximately in the middle of April, the mean SST quite abruptly becomes higher than the mean AT. In 2005 there was a time-lag of 14 days between the rise of the AT to the higher, 'summer', plateau and this switch to positive D_{AT} values. During the years 2006 and 2007, although they were timed later and earlier than in 2005, respectively, these time-lags were very similar, 17 and 14 days, respectively. In the Autumn, the process was reversed again and $D_{AT\text{-}mean}$ values switched to negative 14 days (15 days in 2006) after a sharp drop in the mean AT. It was found that , during 2005, 2006 and 2007, the timing of the start and finish of the summer-temperature plateau was well described by the first and last day the mean temperature had a value of 15°C or higher (see figure A.1). Other 2006/2007 patterns also showed great similarity with the described 2005 patterns (for both mean, maximum and minimum temperatures) and therefore these will not be discussed separately.

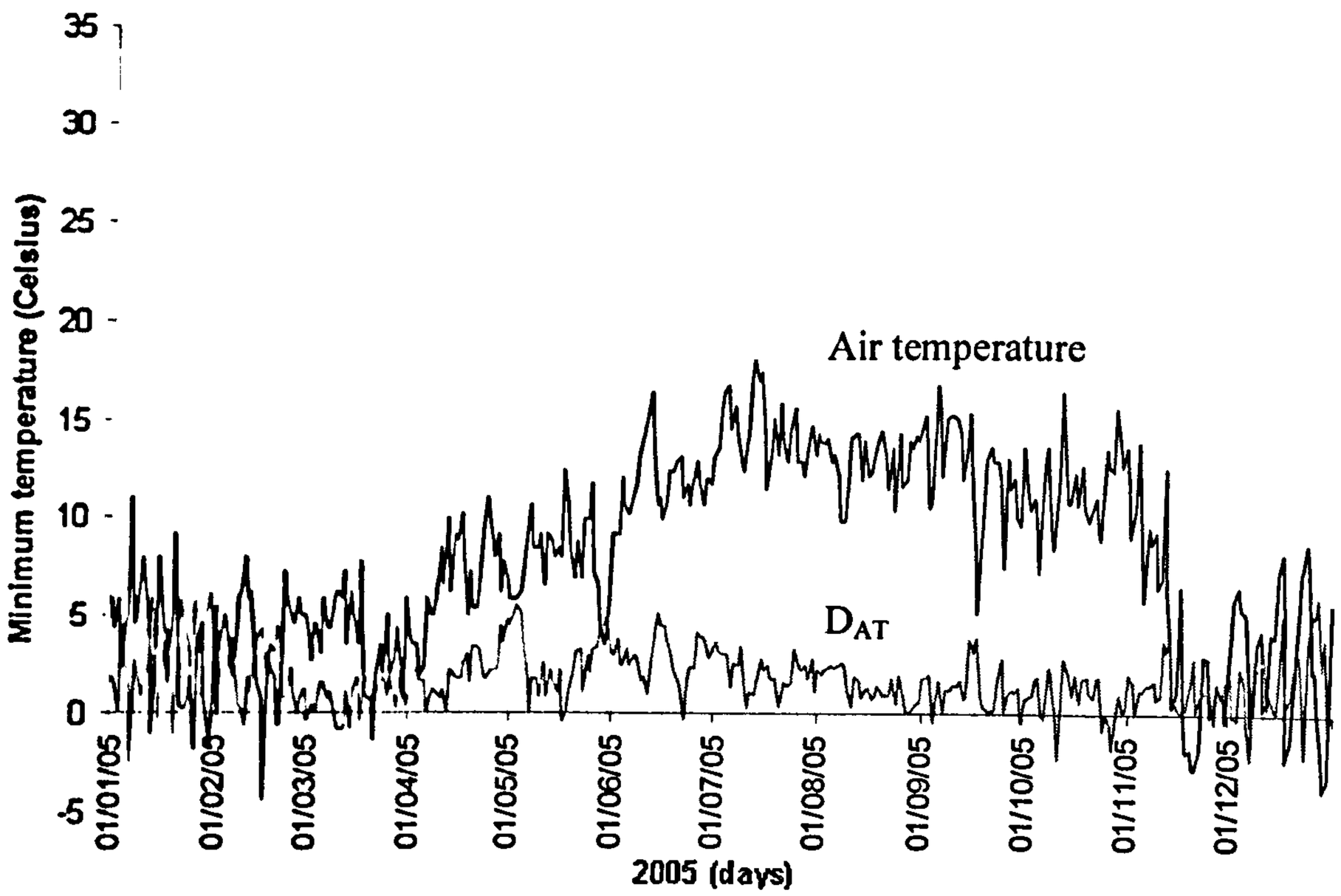
$D_{AT\text{-}mean}$ values were significantly influenced by the month of the year ($F_{(11,335)} = 16.393$, $p \leq 0.001$). The months May, June, July, August and September had significantly higher values



A. Mean temperature



B. Maximum temperature



C. Minimum temperature

Fig.A.1 (A-C) Mean, maximum and minimum air temperature and the corresponding difference between mean, maximum and minimum soil and air temperatures, 2005. In all three graphs the upper line represents the air temperature while the lower line represents the difference between soil and air temperatures (D_{AT}). A negative data point for the latter lines means that the air temperature is higher than the soil temperature.

than all other months ($p \leq 0.036$) while not significantly differing from each other ($p \geq$ 0.654). Differences between the months January to April and October to December were also not significant ($p \geq 0.084$). Test statistics of further tests on the clusters May-September and January-April+October-December, and the regression equations, can be found in table A.1. The regression plots can be found in figure A.2. Mean air temperature was a significant predictor of $D_{AT\text{-}mean}$, whereas rainfall, after controlling for the effect of mean air temperature, was not.

Maximum temperature

The maximum AT measurements, and the corresponding $D_{AT\text{-}max}$ values, are given in figure A.1B. The patterns detected very closely reflected those described under Mean temperature but, as expected, between-day fluctuations in maximum temperature are greater than in mean temperature. Also, the switch from maximum soil temperature being colder than maximum air temperature to the soil being warmer, which takes place at the same time as for mean temperature, is more acute. The time lags described under mean temperature were also found for maximum temperature (14-16 days in the Spring and 14 and 15 days in the Autumn). The timing of the summer-temperature plateau, which determined the timing of the switch, was well described by the first and the last time the maximum temperature had a value of 20°C (figure A.1).

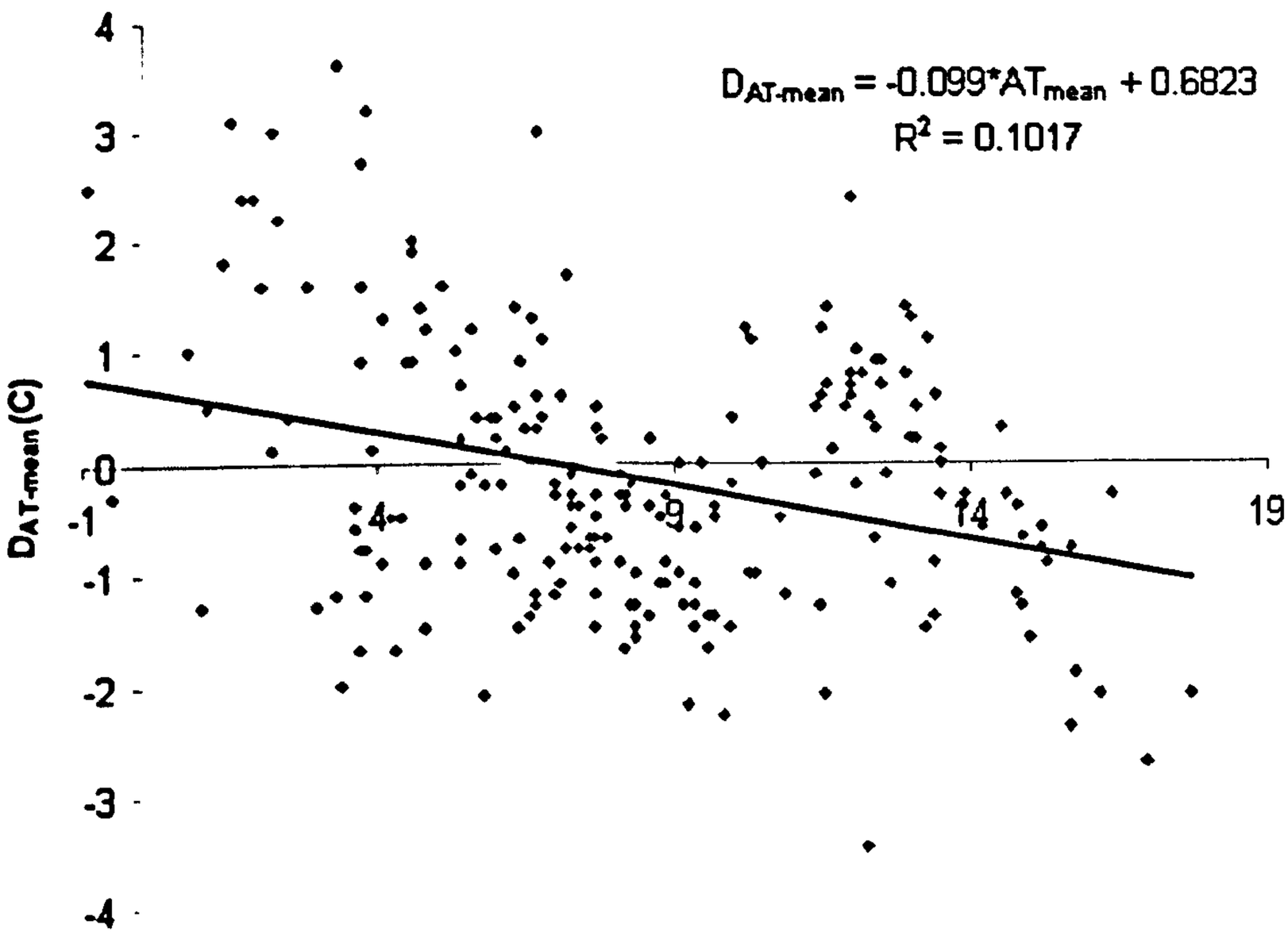
Like $D_{AT\text{-}mean}$, $D_{AT\text{-}max}$ was significantly influenced by the month of the year ($F_{(11,335)}$= 42.646, $p \leq 0.001$). The 5 months May-September were not statistically separated ($p \leq$ 0.009) while their soil temperature values were significantly higher than those of all other months ($p \geq 0.150$). Some of the months January-April and October-December differed significantly but no month or group of months differed significantly from the rest and there was a large overlap in the homogenous subsets of months. As shown in table A.1 AT proved a significant predictor of SST in the months January-April+October-December but not for the months May-September, in which fluctuations in $D_{AT\text{-}max}$ were relatively large. Adding rainfall to the model did not add any significant predictive value. It appears that, during the months May-September, there is no better estimation of $D_{AT\text{-}max}$ than the mean value of 2.0 (range -3 to 6.4).

	Months	Factor	Statistic	Regression equation
Mean temperature	Jan-Apr + Oct-Dec	AT	$F_{(1,212)}$ =1.663, p= 0.042	$D_{AT\text{-}mean}$ = -0.099 *AT + 0.6823 (equation A.1)
	May-Sept	AT	$F_{(1,153)}$=1.812, p= 0.006	$D_{AT\text{-}mean}$ = -0.1029*AT + 2.9235 (equation A.2)
	May-Sept	Rain	$F_{(2,153)}$= 0.001, p= 0.970	N/A
Maximum temperature	Jan-Apr + Oct-Dec	AT	$F_{(1,212)}$= 2.162, p ≤ 0.001	$D_{AT\text{-}max}$ = -0.1147*AT - 0.5708 (equation A.3)
	May-Sept	AT	$F_{(1,153)}$=0.676, p=0.510	N/A Flat rate correction: + 2.0
	May-Sept	Rain	$F_{(2,153)}$= 0.075, p=0.785	N/A
Minimum temperature	All months	AT	$F_{(1,364)}$ = 1.304, p= 0.041	$D_{AT\text{-}min}$ = -0.0698*AT + 2.3507 (equation A.4)
	May-Sept	AT	$F_{(1,153)}$=1.623, p=0.018	N/A
	May-Sept	Rain	$F_{(2,153)}$= 0.973, p= 0.403	N/A

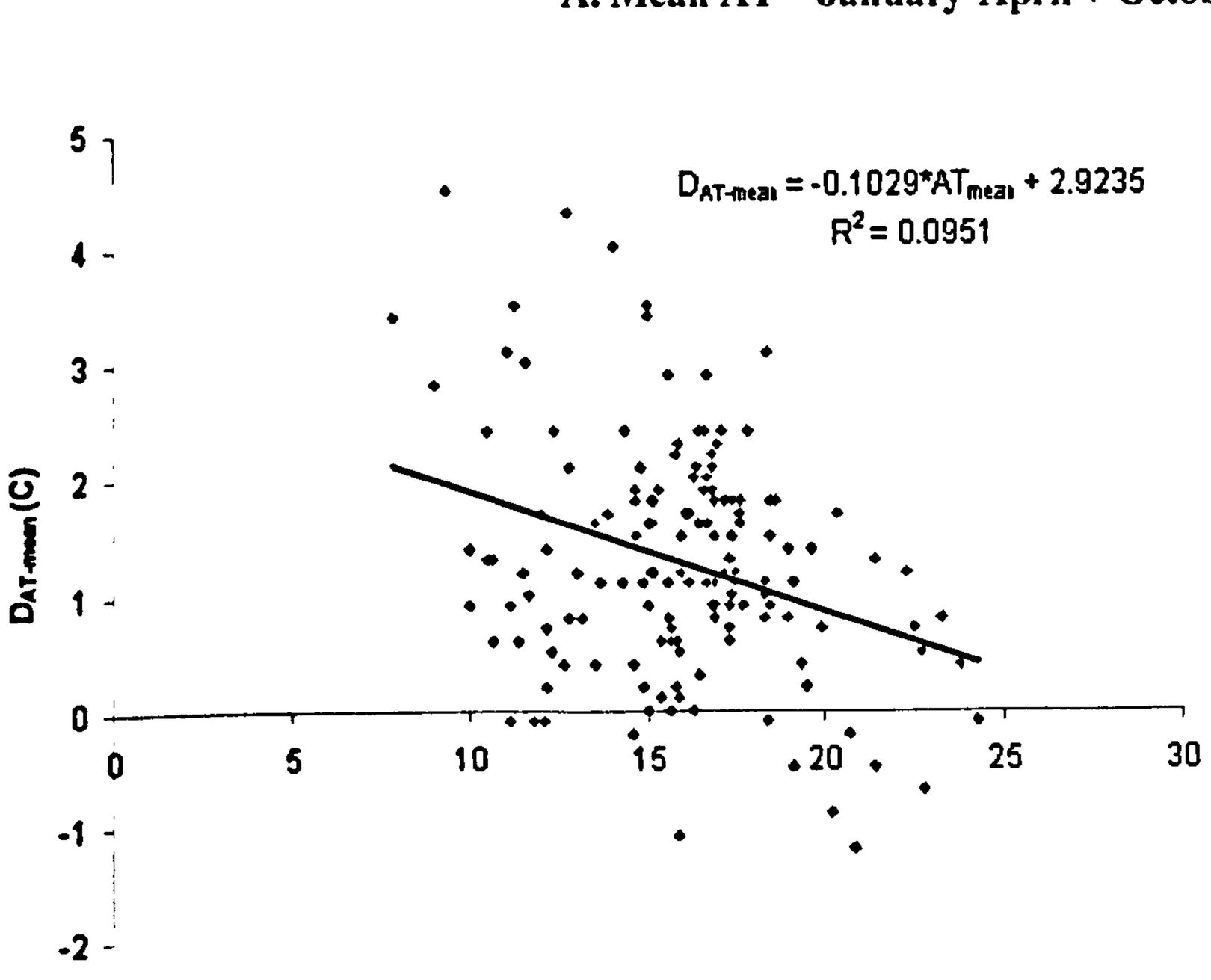
Table A.1 Test statistics and regression equations for the influence of mean, maximum and minimum air temperature (AT) and rain on the difference between these temperatures and mean, maximum and minimum soil surface temperatures at different months of the year. The test statistic given for the factor rain describes the significance of the contribution of rainfall to the description of soil surface temperature (SST), after controlling for the effect of AT. D_{AT} = correction value to be added to AT to get to SST.

Minimum temperature

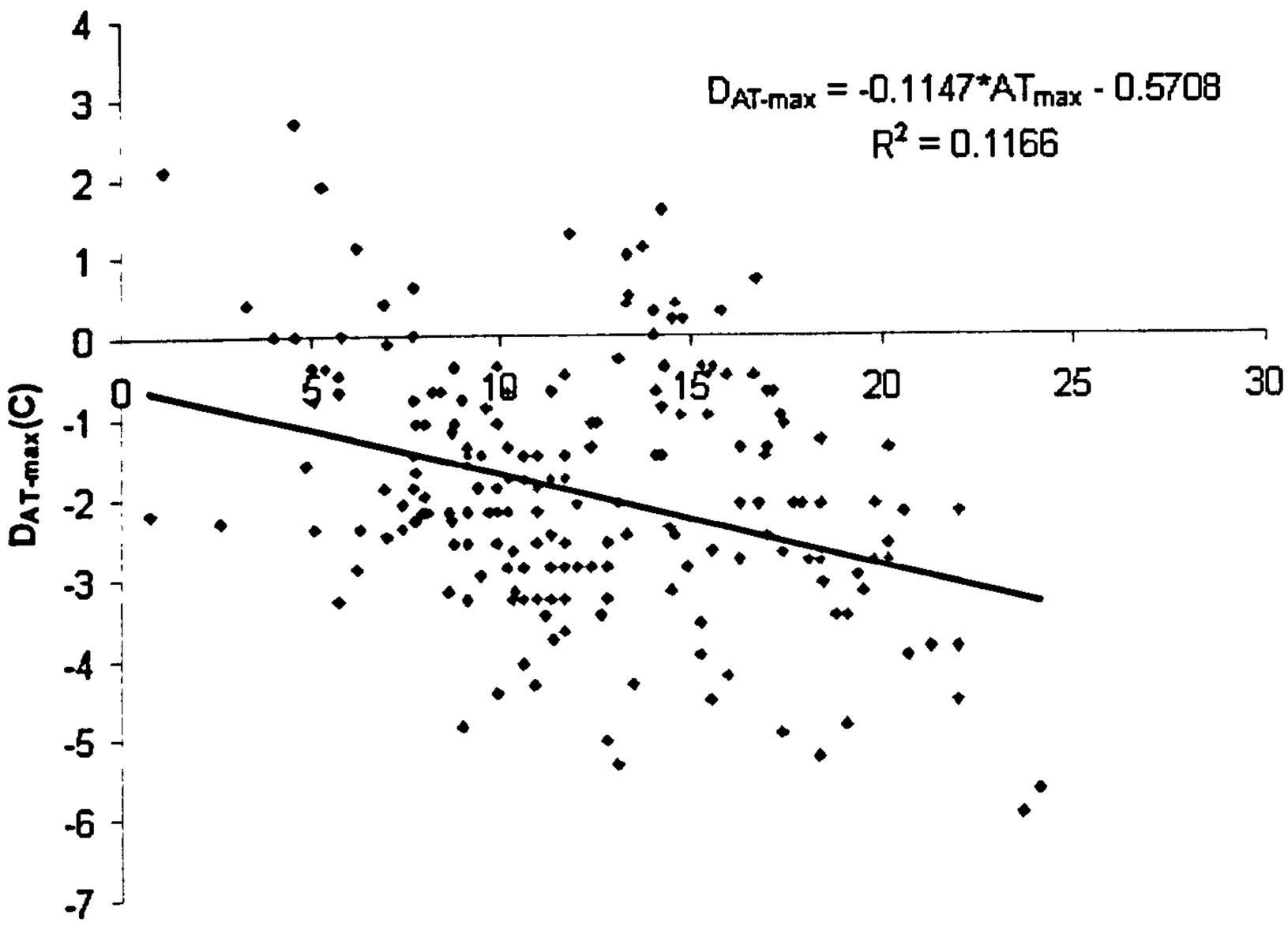
As figure A.1C shows, the difference between minimum SST and AT has a positive value almost throughout the year. Although significant differences between months were found



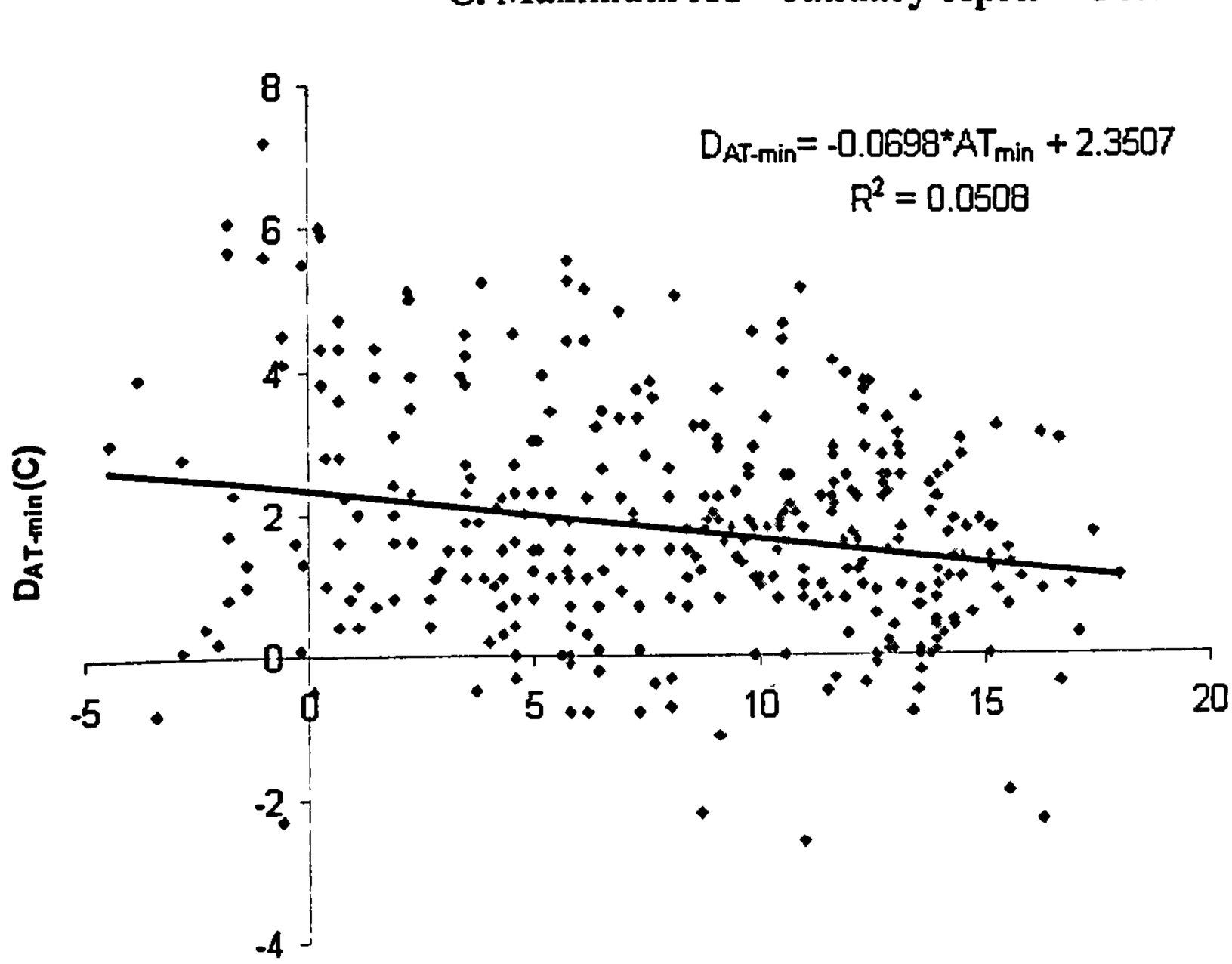
A. Mean AT – January-April + October-December



B. Mean AT- May-September



C. Maximum AT - January-April + October-December



D. Minimum AT- January-December

Figure A.2. (A-D) Regression plots of D_{AT} values on Air Temperature. C = °Celsius.

($F_{(11,335)}$= 6.099, $p \leq 0.001$) no single month, or cluster of months, differed from all others and, of the homogenous subsets, at least 6 months overlapped with other months. When all days of all months were used in the regression minimum AT proved to have significant predictive value of the difference between minimum SST and AT (see table A.1 and regression figure A.2). Again, adding rainfall to the model did not increase this predictive value.

Discussion

In general, for mean and maximum soil and air temperatures it was found that the air was warmer during the coldest months of the year whereas the soil surface was warmer during the warmest months of the year. However, for minimum temperatures no such differences were detected and the minimum soil temperature always had a higher value than the minimum air temperature. The correction of minimum AT to minimum SST could therefore, throughout the year, be described by a single regression equation. Minimum soil temperatures appear to follow AT trends more closely at higher temperatures than at lower temperatures. The slope of the regression equation is such that, over the spectre of minimum AT values encountered in the field, only one or two °C precision is added compared to correcting by the mean value of approximately +2 °C. However, as shown in chapter 7, trichostrongyloids like *Nematodirus battus* are very sensitive to a few degrees Celsius difference in temperature and these may make the difference between hatching or not hatching. In this respect it is also worth noting that the slope of the regression line of the maximum temperature, during the early spring, is greater than that of the minimum

temperature. The effect of these slopes is such that, at higher AT values, the difference between day and night temperatures is somewhat increased and this may again have important implications for the correct prediction of the ability of *N. battus* to hatch in the spring. In terms of climate change this finding may also have important implications for this parasite, as it seems that the soil may exacerbate small increases in day-night AT fluctuations.

When converting mean and maximum air temperatures to mean and maximum soil temperatures it appears no simple single value correction can be applied throughout the year. The value to be added depends both on the month of the year and the value of AT itself. Two clear clusters of months could be identified. In 2005 the switch of negative $D_{AT\text{-}mean}$ and $D_{AT\text{-}max}$ values to positive ones happened exactly at the start of May. Similarly, the reverse happened at the start of October. In other years these switches were timed differently but always approximately 14 days after the air temperature had shown its first sharp incline/decline from the winter and summer plateau levels, respectively. It appears that the winter/autumn/early spring correction factors can be described by equations A.1 and A.3 (table 1), switched to equation A.2 and correction factor +2.0, respectively, 14 days after the first significant AT increase. 14 days after the first sharp autumn decline in AT corrections factors are then once again described by equations A.1 and A.3.

In contrast to minimum soil temperatures, in the early spring, maximum SST do resemble maximum AT better at lower temperatures than at higher temperatures. Therefore, under climate change scenarios, if both maximum and minimum AT increase during the winter,

early spring or autumn the maximum SST is not expected to follow suit as closely as the minimum temperature.

Remarkably, very clearly no effect of rainfall on D_{AT} could be detected. For daily maximum and minimum temperatures it could be argued that the effect of rainfall would very much depend on the timing of the cloudburst but the fact that also no effect was found on $D_{AT\text{-}mean}$ strongly suggests that there indeed is no measurable effect.

Describing D_{AT} as a function of AT it appears to be a simple method for AT-SST conversion that can be applied to warmer regions and colder regions alike.

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